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State-of-the-art technology for device-based and cell/tissue-based approaches

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Abstract

Artificial organs - State-of-the-art technology for device-based and cell/tissue-based approaches

The challenge of treating diseased or failing human organs has been the driving force behind an increasing number of research programmes in recent years. Increased attention is being given to the potential of medical devices constructed from non-living materials as well as to applications utilizing living cells or tissues. Such products can serve as a bridge to transplant or serve as long-term chronic support systems. Artificial organs that can fully replace a failing organ are not yet commercially available.

This RIVM report provides a comprehensive overview of the development of both medical device-based and cell/tissue-based solutions for (partly) failing organ systems of the heart, lungs, liver, kidney, pancreas, bladder and bowel. These solutions are assessed for both total organ replacement and as technologies supporting or repairing damaged organs.

Established medical devices functioning as a bridge to transplant or as long-term chronic support systems currently exist for the heart, kidneys, pancreas and bladder. Clinical trials are ongoing with a total artificial heart device and with heart, liver and kidney support devices.

Cell/tissue products are not yet commercially available, although clinical trials in this field are ongoing for the heart and kidney. The results of research, including small-scale clinical trials, on the so-called bioartificial liver and pancreas, based on the use of porcine cells, were promising. However, the research on such bioartificial organs is currently banned in the Netherlands and many other countries for ethical and safety reasons.

Other applications for total artificial organs are not expected to enter clinical trials within the next 5 or even 10 years. Since Dutch academic research groups and clinicians are working on cutting-edge technology in this field, it can be expected that applications in Dutch clinics will closely follow international developments.

Keywords: artificial organs, medical devices, cell therapy, tissue engineering, regenerative medicine

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Rapport in het kort

Artificiële organen – Stand der wetenschap voor benaderingen met medische hulpmiddelen en met cel/weefsel-producten

Onderzoek naar oplossingen voor zieke of falende organen van de mens is de laatste jaren sterk in opkomst. Het gaat hierbij zowel om medische hulpmiddelen van niet-levende materialen als om toepassingen met levende cellen of weefsels. Dergelijke producten kunnen de wachttijd tot een transplantatie overbruggen, of de werking van een orgaan gedurende lange tijd ondersteunen. Kunstorganen die falende organen volledig kunnen vervangen zijn nog niet op de markt.

Dit RIVM-rapport geeft een overzicht van de ontwikkeling van zowel medische hulpmiddelen als cel/weefselproducten die (gedeeltelijk) falende orgaansystemen ondersteunen, repareren of vervangen. Het gaat daarbij om het hart, de longen, de lever, de nieren, de pancreas, de blaas en de darmen.

Voor het hart, de nieren, de pancreas en de blaas bestaan medische hulpmiddelen als overbrugging naar een transplantatie of als langetermijnondersteuning van het orgaan. Er vinden klinische studies plaats met een totaal kunsthart, en met ondersteunende hulpmiddelen voor hart, lever en nieren.

Cel/weefselproducten zijn nog niet op de markt. Klinische studies vinden plaats voor het hart en de nieren. Onderzoek naar zogenoemde bioartificiële levers en pancreassen op basis van varkenscellen leek veelbelovend, ook in kleinschalig klinisch onderzoek. Vanwege ethische en veiligheidsredenen is dit momenteel in Nederland en vele andere landen verboden.

Andere concrete toepassingen voor totale kunstorganen worden de komende vijf tot tien jaar niet in klinische studies verwacht. Aangezien Nederlands onderzoek op dit gebied mondiaal vooraan meeloopt, zullen eventuele internationale doorbraken wel snel hun weg vinden in ons land.

Trefwoorden: artificiële organen, medische hulpmiddelen, celtherapie, ‘tissue engineering’, regeneratieve geneeskunde

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Contents

Summary

Introduction
Artificial organs can be defined as products that are intended to be used for the (partly) support, replacement or regeneration of diseased, damaged or otherwise not fully functional organs. For patients with severely damaged organs, who are on a waiting list for a transplant organ, the availability of artificial organs could be the only way to survive. One way of creating artificial organs is the use of cell therapy and/or tissue-engineering techniques. Also medical device solutions based on mechanical, optical, electrical, physical or other technological characteristics can be applied, as well as combination products using distinct features from both devices and cell products. In recent years, important innovations have been realized in the field of medical technologies in general, which have also impacted the development of artificial organs.
This report describes the state-of-the-art of both medical device-based and cell/tissue-based solutions for (partly) failing organ systems of heart, lungs, liver, kidney, pancreas, bladder and bowel. The report addresses total replacement of organs, as well as technologies to support or repair damaged organs, but not organ transplantations. Furthermore the report contains an overview of universities, hospitals and commercial organizations working on such technology in the Netherlands. The following paragraphs contain the developments for each organ in summary, followed by an overall conclusion.

Heart
Numerous devices have been developed for mechanical circulatory support in patients with end-stage heart failure. Ventricular assist devices (VADs) are in routine use as a bridge to transplantation, bridge to recovery, and long-term chronic support. The latest generation includes axial and centrifugal flow blood pumps. Also cardiac pacing and defibrillation devices are well established technologies. Currently, one total artificial heart (TAH, i.e. AbioCor™) is being used under the Humanitarian Device Exemption in the USA. The next generation TAH (i.e. AbioCor II) is being developed. It is smaller in size and therefore suitable for more patients. In Europe, the ACcor TAH and the MiniACcor TAH are currently being developed. These devices have been tested in animal studies and circulatory mock loops. In the Netherlands, chronic animal experiments with the MiniACcor are being planned at the Radboud University Nijmegen in cooperation with the Heart- and Diabetes Centre in Bad Oeynhausen (Germany) and the clinic for Thoracic- and Heart Surgery in Nijmegen.
Cell therapy of the heart seems to be the most abundantly practiced cell therapy in the clinic. Several approaches regarding cell source and cell delivery are being evaluated, of which the infusion and injection of bone marrow derived stem cells are the most abundant. This also accounts for the clinical studies that are performed in hospitals in the Netherlands. Many studies are ongoing and more are expected to be initiated in the future. In particular, a large multicentre trial in ten large Dutch hospitals is going on, as well as separate trials in Rotterdam and Leiden. In 2006, a clinical trial at the Medical Centre of Twente was cancelled by the Dutch Central Committee on Research Involving Human Subjects (CCMO) after 8 of 10 patients had been treated with injected stem cells. The trial had been initiated without approval of the CCMO. The manipulation of the stem cells before implantation was performed by the Dutch company Cells4Health BV (Leuvenheim, The Netherlands), a specialised company for the harvesting, treatment and storage of stem cells derived from both umbilical cord blood and bone marrow. Currently, the company is still offering a treatment of

acute myocardial infarction called Health-Cardiac MI. This treatment is performed in collaboration with the University Hospital in Gaziantep in Turkey. As stated on the webpage of Cells4Health, the first patient (candidate for heart transplantation) has already been treated. This treatment is not acknowledged by the Dutch National Health Service and is therefore not covered by the health insurers and can not be performed in the Netherlands.
Unfortunately, no major conclusions can yet be drawn based on the published studies conducted in the Netherlands. Based on the results obtained worldwide, feasibility and relative safety of cardiac cell therapy has been demonstrated. However, also large variation in efficacy is obtained, ranging from negligible to marginal. Although it can be considered that the proof-of-concept has been demonstrated, there is a need to explore and clarify the mechanism of action in order to improve efficacy. It can therefore be expected that many clinical studies as well as non-clinical studies will remain to be conducted in the coming years throughout the world, including the Netherlands.

Lungs
The natural lung represents a remarkable organ for gas exchange, and developing an artificial lung that approaches the gas exchange powers of the natural lung is a significant engineering challenge. Current hollow fiber blood oxygenators, as used in cardiopulmonary bypass, have membrane areas ranging from 1 to 4 m^2 that are packaged much less compactly than in the natural lung, with a surface area to blood volume ratio 10 times less than in the natural lung. The effective distance that gas diffuses between blood and gas flow pathways in artificial lungs is approximately 10–30 μm, an order of magnitude greater than in the natural lung. Thus, even with using 100% oxygen gas, artificial lungs currently used or under development aim at gas exchange levels that can only support resting metabolic needs in patients. None of the artificial lungs described in this report make an attempt to mimic any of the other functions or properties of the lung. The non-cell artificial lungs currently under development derive directly in a conceptual sense from the hollow fiber membrane and membrane module technology used in traditional clinical blood oxygenators.
Regarding the cell-based solutions, several approaches are being developed worldwide. These can be divided into the following categories: I) Targeted activation or administration of endogenous stem cells, II) Creation of pulmonary tissue constructs in vitro, III) Biohybrid lung that combines a medical device with living cells. All of these approaches are still in the research phase, although it has been reported that a cell-coated tracheal substitute has been applied in one patient (Germany). In the Netherlands the development of constructs with living cells to reconstruct, repair or replace pulmonary tissue and function has yet to be initiated. Therefore, it can be concluded that it is not expected that cell-based treatment of the lung will be applied in the Dutch clinics in the coming 5-10 years.
The challenge of biocompatibility inherent in making microvascular-scale blood channels with an extensive blood contact area, that is non-thrombogenic and non-inflammatory, may require the use of endothelial cells, perhaps genetically engineered for enhanced performance or for the robustness required in the application. Significant advances in tissue engineering, biomaterials, microfabrication, and bioengineering will all need to be harnessed for the technological development of future artificial lungs. Artificial lungs that allow patients any significant level of increased metabolic activity are not on the immediate horizon. At the same time, the need for artificial lungs in the distant future may be eclipsed by significant advances in regenerative medicine that enable tissue repair and regeneration of failing lungs.

Liver
Enthusiasm for liver support devices, particularly cell-based biological systems and albumin dialysis, has increased over the last decade resulting in considerable clinical activity both

within and without the construct of clinical trials. Most data have been generated on patients with acute liver failure or on patients with decompensation of chronic liver disease, often referred to as acute-on-chronic liver failure. In clinical use for acute liver failure, bridging to liver transplantation is a more realistic goal rather than to transplant-free survival. In acute-on-chronic liver failure the objective of attaining clinical stability with treatment appears more achievable.
Currently, there is no single artificial organ or device capable of emulating all the functions of the liver. Some functions related to removal of toxic substances can be emulated by liver dialysis, charcoal hemoperfusion or plasma exchange, experimental treatments for liver failure. These methods have not yet been shown to improve the survival of patients with liver failure, although hemodialysis did work well on renal failure associated with liver failure. A small clinical trial (n=5) using a slow plasma exchange in combination with high-flow continuous hemodiafiltration showed some promise. The most promising medical device approaches at this moment are SPAD (single pass albumin dialysis) and MARS (molecular adsorbent recycling system), which combines conventional dialysis with albumin dialysis. Both approaches are still in an experimental phase and the future prospects rely on the performance of adequately powered randomized controlled trials. In the Netherlands artificial liver devices are currently not used on patients with acute or acute on chronic liver failure, although early stages of clinical studies on MARS therapy are being explored.
Medical device-based artificial liver support systems have a beneficial influence on the neurological state of patients, but do not improve survival. More beneficial effects have been expected from systems that bring the blood of the patient in contact with living liver cells, or: bioartificial liver systems (BAL). The cell activity can then contribute to the compensation of the failing patient liver, by e.g. detoxification, biosynthesis and biotransformation. The BAL may be developed as a bridge to transplant for patients suffering from acute-on-chronic liver failure or for some patients as a bridge to recovery.
The BAL based on porcine hepatocytes, is the most extensively evaluated type of biological device. A sizeable clinical trial failed to demonstrate efficacy, but secondary analyses suggest it would be unwise to assume that futility had been established with this device. Concern exists about the possible adverse immunological reactions towards the animal cells in the BAL, the transfer of cells into the patient which may lead to tumors and the transfer of retroviruses to the patient that may eventually pose a public health hazard. The further research of BAL devices incorporating porcine cells is banned in the Netherlands as well as in many other countries.

Kidneys

The kidney has multiple functions. Next to the excretion of waste substances, it also provides the overall important homeostasis of the blood through a sophisticated system of hormone excretion and re-absorption of minerals, water and proteins. Current hemodialysis therapy, which is the standard treatment for patients with end stage renal disease, does not provide the latter and, as a consequence, is associated with considerable morbidity and mortality. Two systems are under development that are expected to improve the renal replacement therapy and may lead to higher survival rates in patients that are waiting for kidney transplantation.
One system uses a pure medical device-based approach. It mimics the excretion and reabsorbtion function of the kidney by means of double filtration membranes. One membrane functions like a ‘classic’ hemofiltration unit. The second membrane is designed to reabsorb substances from the hemofiltrate, which are lost in the ‘classic’ hemodialysis. The selectivity of the membranes can be vastly improved by new production techniques. Smart nano-membranes can be designed to selectively pass molecules, not only based on the size of the molecules, but also on dielectric properties of the molecule.

A second possibility is formed by the use of living cells or tissues. The cell-based approaches that are currently in development can be divided into the following categories: I) Repair of the kidney by infusion of stem cells, II) Transplantation of fetal kidney tissue, III) Use of extracorporeal cell-coated devices, IV) Use of in vivo renal cell-coated matrixes. The most promising approach for the near future is likely to be the use of extracorporeal cell-coated devices, since this is the only approach that has entered clinical trials worldwide. This principle is based on a tissue-engineered bioartificial bioreactor that consists of a confluent layer of cultured proximal tubule cells seeded on the luminal side of multiple polysulfone hollow-fibers. This bioreactor is combined with a conventional hemofilter and acts to mimic the process of tubular reabsorption. The results obtained in the clinical trials (USA) indicate that this approach is effective. In the Netherlands a similar approach is aimed to be developed as part of the BioMedical Materials Program (BMM). Although the ultimate goal is to develop an implantable bioartificial kidney, the first big milestone will be the creation of such an extracorporeal artificial bioreactor. Nevertheless, this program has just started and clinical studies in the Netherlands with cell-based artificial kidneys to repair, replace or reconstruct renal function are not expected in the coming 5-10 years.

Pancreas

A (bio-)artificial pancreas would improve the quality of life of insulin dependent patients and would have medical benefits. For over 40 years now, studies have been performed on the development of a closed-loop glucose measurement and insulin delivery system. In the last decennia progress has been made in the development of essential components: glucose monitors and insulin pumps. Both are commercially available, including dose advising algorithms and data management options, and the application possibilities become more sophisticated year after year. However, fully closed-loop systems are still not reliable and sufficiently accurate to be marketed. This is mainly due to problems with long term glucose measurement and to the complexity of dose controlling algorithms that have to respond to many different physiological circumstances. In the Netherlands, 15 patients are using a continuous glucose monitor in combination with an insulin pump. Furthermore, in 2007 a user evaluation study has started in 12 hospitals such a system in which the glucose monitor can communicate with the insulin pump. With these systems the patient still has to decide on the insulin dosing, based on the glucose levels displayed on the device.
Cell-based therapeutic options include the use of stem cells and the construction of a bio-artificial pancreas (BAP). Therapies for diabetes based on stem cells have yet not reached maturity and are still in the laboratory phase. BAPs can be intravascular or extravascular. The intravascular devices bear the risk of coagulation and thrombus formation and are currently not the approach of first choice. The extravascular devices do not present these problems and especially microcapsular devices have been studied extensively. Clinical investigations with BAPs are scarce, but have been carried out in the USA, Canada, Italy, Mexico and Russia. The latter two studies used porcine cells, which is not acceptable for ethical reasons in many countries including the Netherlands. To the best of our knowledge, clinical applications using a cell- or tissue-based artificial pancreas are currently not performed in the Netherlands.

Bladder

Several implantable medical device and (surgical) techniques are available for the treatment of bladder dysfunction. Some of these devices and techniques have proven to be successful, most notably sacral root stimulation, sacral nerve neuromodulation, and artificial urethral sphincters. Far-reaching surgical procedures (e.g., rhizotomy), technical failures (in case of artificial urethral sphincters), and the lack of selective neural activation must be overcome before these implantable medical devices can gain more widespread use. Many details

regarding these techniques have yet to be elucidated. On the horizon are new and emerging technologies (e.g., BIONs, optical stimulation) that could contribute to accomplish improved bladder control. In the Netherlands, academic centres in Nijmegen, Utrecht, Rotterdam and Maastricht are involved in the clinical application of medical devices for the recovery of bladder function.
Several cell-based approaches for bladder repair, reconstruction and replacement are being explored worldwide. These approaches do not comprise the development of the bladder as a whole organ, but are focused on specific parts or diseases of the bladder: I) Recovery of the urethral sphincter, II) Treatment of vesicoureteral reflux III) Recovery or replacement of the bladder wall. Clinical trials have been performed or initiated in all of these groups, although to our knowledge none of these studies are currently performed in the Netherlands. Nevertheless, a European program in which the Netherlands is playing a key role and that most probably includes the development of a cell coated artificial bladder wall has been initiated in January 2007. Although it is difficult to predict, it cannot be excluded that early clinical trials using cell-coated artificial bladders will be initiated in the coming five years.

Bowel
Recent developments in sacral nerve stimulation, artificial bowel sphincter procedures, and dynamic graciloplasty are considered to be promising. Enthusiasm for any new technique often leads to overemphasis of the outcomes, and early reports are usually good. Outcomes can deteriorate with time and long-term results do not correspond to initial encouraging data such as for instance in case of the artificial bowel sphincter or dynamic graciloplasty. Both methods are technically demanding, with considerable morbidity, and substantial learning curve. Despite these obvious disadvantages, both artificial bowel sphincter and dynamic graciloplasty remain attractive to colorectal surgeons because once successful, they provide outstanding and long-lasting improvement of bowel function and quality of life. Unfortunately, these procedures require special equipment and their utility is limited because there is high morbidity to consider, which discourage coverage by health care insurers. Tissue engineering approaches to create novel bowel tissue are currently still at the stage of proof-of-concept in small experimental animals. Promising research is going on for both small and large intestine. However, no clinical studies are expected in the near future.

Conclusion
In the field of artificial organs, many research programs are being performed on all major organ systems. However, only a limited number of the approaches have reached the phase of application in clinical use. Established organ support devices exist as a bridge to transplant or long-term chronical support for the heart, the pancreas and the bladder. Clinical trials are going on with a total artificial heart device, liver and kidney support devices and with cell therapy for the heart and a tissue-engineered bioreactor combined with dialysis functions to replace failing kidneys. The bioartificial liver and pancreas based on the use of porcine cells have shown some promise, also in previous small-scale clinical trials, but are currently banned in the Netherlands and many other countries for ethical reasons. For lungs, liver, kidneys, bladder and bowel both device-based and cell/tissue-based solutions are in various promising stages of development which are, however, not expected to enter clinical trials within the next 5 or even 10 years. Dutch academic research groups and clinicians are working at the front line in this field, implying that applications in Dutch clinics will closely follow international developments.

1. Introduction

1.1 Background

The early years of the 21st century show an acceleration of the introduction of innovative medical technologies. This revolution in the capabilities of medical technologies has been attributed to the coincidental emergence of several areas of science and technology which, when combined, will act as protagonists and strengthen each other. The most important areas involved are the biological sciences, nanotechnology, cognitive sciences, information technology and materials science. Many innovations are already resulting from the combination of these fields. Furthermore, new generations of medical technology products are being produced that increasingly cut across traditional demarcation boundaries such as medical devices, pharmaceutical products or human tissues. The trend to combine different technologies and the crossing of borders between traditional categories of medical products is commonly referred to with the term 'converging technologies'. An important category of products that is benefiting from such technological progress are the artificial organs. We use the following definition:
Artificial organs are products that are intended to be used for the (partly) support, replacement or regeneration of diseased, damaged or otherwise not fully functional organs.

For patients with severely damaged organs, who are on a waiting list for a transplant organ, the availability of artificial organs could be the only way to survive. One way of creating artificial organs is the use of cell therapy and/or tissue engineering techniques. Also new medical device solutions based on mechanical, optical, (electro-)physical or other technological characteristics are being developed, as well as combination products using distinct features from both devices and cell products.
The Dutch Inspectorate for Health Care is aware that artificial organs are being developed at increasing speed. Since it is their role to keep a watching brief on the safe application of health care, they need to be prepared for the introduction of such new technologies. Therefore, they have commissioned the Dutch National Institute for Public Health and the Environment (RIVM) to provide an overview of the current state of affairs.

1.2 Aim

The aim of this report is to describe the state-of-the-art with regard to the development of artificial organs. Furthermore, universities, hospitals and commercial organisations working on such technology in the Netherlands will be identified.

1.3 Scope

The report describes both medical device-based and cell/tissue-based solutions for (partly) non-functional organs. This includes total replacement of an organ, as well as technologies to support or repair damaged organs. Organ transplantation is not included, nor repair of tissues

like skin, cartilage or bone. The report is restricted to the following major organ systems: heart, lungs, liver, kidney, pancreas, bladder and bowel.

1.4 Methodology

This report was based on literature searches, internet searches, electronic newsletters and proceedings of conferences. Literature was identified from several sources including electronic databases and cross-checking of reference lists. Electronic databases consulted for scientific literature were Scopus™ (Elsevier BV) and Medline/PubMed (US National Library of Medicine). Internet searches were performed starting from the search engine Google (www.google.com). Product information was obtained using manufacturers' websites. Work in progress at universities, hospitals and companies was obtained from the relevant websites as well. In addition, interviews were held with key stakeholders in the Netherlands.

2. Heart

The heart's primary function is to pump blood to all parts of the body, bringing nutrients and oxygen to the tissues and removing waste products. When the body is at rest, it needs a certain amount of blood to achieve this function. During exercise or times when greater demands are placed on the body, more blood is required. To meet these variable demands, the heartbeat increases or decreases, and blood vessels dilate to deliver more blood or constrict during times when less blood is required. The heart's structure has four chambers with one-way flaps called valves (Figure 2.1). The atria are the upper chambers and they receive blood that is being returned to the heart. The right atrium receives blood with little oxygen because the blood has already circulated throughout the body delivering oxygen and nutrients. The left atrium fills with newly oxygenated blood returning from the lungs. When the atria contract, they push the blood through valves (tricuspid and mitral) into the relaxed ventricles. When the ventricles contract, the right ventricle pumps blood through the pulmonary valve into the lungs. The left ventricle pumps blood through the aortic valve to the body, including the heart (through coronary arteries). This continuous cycle of synchronized contractions is driven by the heart's electrical system [www.medtronic.com].

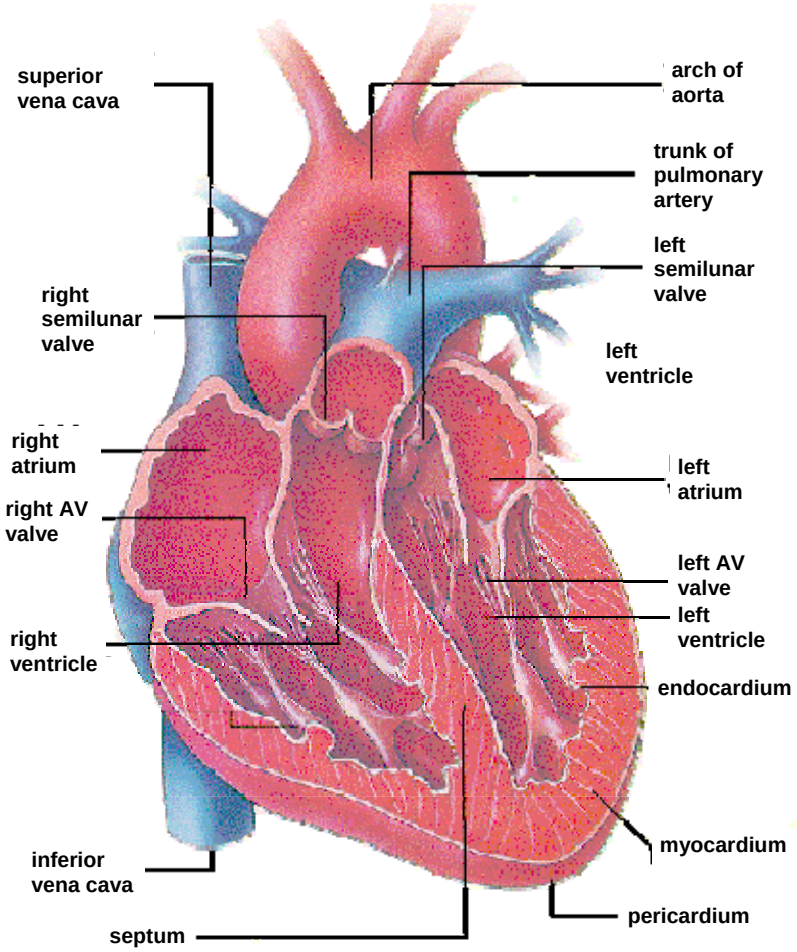

Figure 2.1 Anatomy of the heart [nl.wikipedia.org].

Disorders and current treatment
When a person is diagnosed with heart failure, it means that the heart is not working as efficiently as it should. Heart failure may be reversible, and people may live for many years after the diagnosis is made. Heart failure may occur suddenly, or it may develop gradually. When heart function deteriorates over years, one or more conditions may exist. The strength of muscle contractions may be reduced, and the ability of the heart chambers to fill with blood may be limited by mechanical problems, resulting in less blood to pump out to tissues in the body. Conversely, the pumping chambers may enlarge and fill with too much blood when the heart muscle is not strong enough to pump out all the blood it receives. In addition, as the architecture of the heart changes as it enlarges, regurgitation of the mitral valve may develop, making the heart failure even worse. There are an estimated two million people in the United States alone with heart failure. In the Netherlands, about 170,000 people suffered from heart failure in 2000. The incidence of chronic congestive heart failure (the number of new cases developing in the given population each year) has increased in recent years. This is possibly a result of the overall decline in deaths from coronary (ischemic) heart disease, an improvement attributed to medical advances and the fact that people are living longer. The most common cause of congestive failure is coronary artery disease (narrowing of the arteries supplying blood to the heart muscle). Although coronary disease often starts at an early age, congestive failure occurs most often in the elderly. Among people more than 70 years old, about 8 out of 1,000 are diagnosed with congestive heart failure each year. The majority of these patients are women, probably because men are more likely to die from coronary artery disease before it progresses to heart failure. Hyperthyroidism, arrhythmia and various abnormalities of the heart valves (particularly aortic and mitral) are among the other disorders that can cause heart failure. In addition, viral infection or inflammation of the heart (myocarditis) or primary heart muscle disease (cardiomyopathy), and in rare instances, extreme vitamin deficiencies, can result in heart failure.
When a patient is diagnosed as having heart failure, the first treatment is often by means of drugs. The applied types of drugs depend on the type of heart disease and include diuretics (influences blood volume and thus the heart's workload) and drugs that influence the pumping action by either strengthening the heart's pumping action or stimulation of vasodilatation. In other cases surgery may be the best treatment of choice. When heart failure is due to valvular disease, surgical implantation of an artificial heart valve or valve repair may alleviate the problem. Surgery may also be helpful in correcting congenital heart defects that can lead to heart failure. Coronary artery bypass graft surgery and catheterization using a balloon to flatten fatty deposits (called angioplasty) are among the therapeutic techniques used to prevent and treat heart failure caused by occluded, or blocked, arteries. In recent years, the placement of a stent - either a bare metal stent or a drug eluting stent - after angioplasty has become a procedure of choice. Heart transplants are a last resort in treating severe heart failure caused by diseased heart muscle.

2.1 Medical device-based approach for function recovery

The most important two classes of active implants used to assist or replace the heart function are blood pumps and cardiac pacing and defibrillation devices.
A cardiac pacemaker is a small battery-powered device that is implanted permanently into the body intended for heart rhythm control in case the heart's natural pacemaker, i.e. the sinus node, is not functioning properly. A pacemaker is used when the heart beats too slowly (bradycardia) or has other abnormal rhythms (arrhythmias). In some cases, pacemakers are

also used to treat symptoms of heart failure. An implantable cardioverter-defibrillators (ICD) is a device designed to quickly detect a life-threatening, rapid heartbeat. New models of cardiac pacemakers and cardioverter-defibrillation devices enable stored diagnostic information. This information provides crucial data about device and lead function, and arrhythmias discovered with device interrogation. It is invaluable when troubleshooting problems with devices. Better diagnostic data allow for earlier and more accurate identification of device malfunction as well as better arrhythmia management. Additionally, advances have been made in hardware, leads, and better algorithms. An extensive overview of developments in cardiac pacing and defibrillation devices and related risks is provided by Geertsma et al. [2007].

Blood pumps were initially developed for the temporary support for patients with end-stage heart failure until a donor heart could be found. This intended use was termed bridge to transplantation (BTT) [Pennington et al., 1989; Portner et al., 1989]. Currently, blood pumps are routinely implanted in patients who are eligible for transplantation. Prolonged blood pump support in the BTT setting revealed in some patients the propensity of the myocardium to recover, allowing removal of the medical device rather than previously necessary heart transplantation. This remarkable recovery is termed bridge to recovery (BTR) [Goldstein et al., 1998; Westaby et al., 1997]. For those who recover, the necessary duration of the blood pump support varies from days up to one year. The increasing duration of device implantation has led the question whether implantable blood pumps for permanent use are a suitable alternative to heart transplantation, often referred to as long-term chronic support (LTCS) or destination therapy [Goldstein et al., 1998]. This may have the advantage of earlier intervention and rehabilitation of patients with end-stage heart failure, and avoids the risks associated with immunosuppression and organ rejection following heart transplantation. The use of blood pumps as a LTCS is relatively new and uncertainty remains as to the duration of the support possible.

A substantial part of patients requiring long-term left ventricular support have also right ventricular complications which may lead to complete cardiac failure. Left ventricular support is not sufficient in these patients and either transplantation or the use of a permanent heart replacement system is indicated. The information in this report on blood pumps to assist heart function adapted and updated from our prvious report on new and emrging medical technologies [Geertsma et al., 2007].

2.1.1 State of development

Blood pumps can be classified in two main categories:

- The permanent heart replacement system or total artificial heart (TAH) which replaces the explanted natural heart in terms of anatomical placement and function.
- The ventricular assist device (VAD) which is implanted to assist the natural heart leaving the patient's own heart in place and still functioning. VADs support either left ventricle (LVAD) or right ventricle (RVAD), or both as biventricular assist device (BiVAD). VADs have entered the clinical arena as:
  - Displacement (or pulsatile flow) devices or,
  - Rotary (or continuous flow) devices, which are sub-classified in:
    - Axial flow blood pumps,
    - Centrifugal or radial flow blood pumps,
    - Diagonal flow blood pumps or mixed flow systems (mainly used as extracorporeal devices for cardiopulmonary bypass systems).

There are differences in the configuration of the blood pumps in terms of the position of the pump (extracorporeal, paracorporeal or intracorporeal), implantation position (intra-

abdominal, intraperitoneal, or preperitoneal pocket), method of driving the mechanism (pneumatically, electrically, magnetically driven), type of power source (wall-mounted, console-based or battery packs), positioning of the cannulae and leads delivering the power, valve structure, and the nature of the internal surfaces of the devices. Intracorporeal blood pumps are either connected by percutaneous leads through the patient's skin or totally implanted.
In considering pump design theory, axial flow blood pumps generate high flows at low pressure differences, whereas centrifugal flow pumps are capable of producing higher pressures at lower flows. Diagonal flow pumps tend to have the capability of high-generated pressures and high flow rates. Axial flow blood pumps, although far more compact than centrifugal pumps, operate at much higher rotational speeds to produce the desired head pressure and flow. Because of their small size and tubular configuration, axial pumps require less time to implant, thereby decreasing the costs and invasiveness of the procedure. Centrifugal pumps typically weigh more than axial flow pumps, and this may lead to patient discomfort after installation. In addition, axial flow blood pumps generally consume less power, which allow for more compact and lighter power supply components and eventually implantable batteries.
To date, most blood pumps are produced in the USA, but currently some European companies have caught up with continuing technological developments, and achieved important advantages in miniaturisation, magnetic levitation, low power use, and ease of implantation of pumps, e.g. percutaneous catheter delivery of intracardial devices. Furthermore, necessary monitoring and therapeutic interventions remain to be clarified and standardised.
Due to the large number of different pumps, which are already in clinical use or under development, the following section includes only a brief description of a selection of blood pumps. Extracorporeal devices are excluded.

2.1.1.1 Total artificial heart (TAH)

Until recently, only pneumatic total artificial hearts (TAHs) with extracorporeal driving systems have been clinically used including prominent examples such as the Jarvik 7™ of Jarvik Heart, Inc. (New York, NY, USA) and its successor the CardioWest™ temporary TAH of SynCardia Systems, Inc. (Tucson, AZ, USA). These systems have been used as BTT, or in a few cases, as LTCS for patient with end-stage heart failure [Copeland et al., 1989]. Since July 2001, several patients have been implanted with a different type of TAH, a fully implantable prosthetic device.

AbioCor™ TAH and AbioCor II

The AbioCor™ TAH of Abiomed, Inc. (Danvers, MA, USA) is the world's first completely self-contained replacement heart. May 2004 the 14th patient received a prosthetic heart in the USA [Dowling et al., 2004]. The system is designed for LTCS enabling the patient to remain mobile and continue an active/productive lifestyle. After orthotopic implantation the device does not require any percutaneous tubes or wires. Equipped with an internal motor, the AbioCor™ TAH is able to move blood through the lungs and the rest of the body, simulating the rhythm of the heartbeat.
The AbioCor™ TAH consists of both external and internal components. The internal components are the thoracic unit or pump, rechargeable battery, controller and TET coil (Figure 2.2). The thoracic unit consist of an energy converter and two pumping chambers that function as left and right ventricles. The energy converter is situated between the ventricles and contains a high-efficiency miniature centrifugal pump driven by a brushless DC motor. This centrifugal pump operates unidirectionally to pressurize a low-viscosity fluid. A two-

position switching valve is used to alternate the direction of hydraulic flow between left and right pumping chambers. This results in an alternate left and right systole. The rate of the switching valve determines the beat rate of the device. There is a one-to-one correspondence between blood and hydraulic fluid displacement. The displacement of hydraulic fluid to one side results in the creation of a negative pressure in the opposite ventricle. Thus the device is considered an active fill device.
The internal controller, placed abdominally, drives the energy converter in the thoracic unit, monitors the implanted components, and transmits device performance data to a bedside console by means of radiofrequency telemetry. These radiofrequency transmissions from the internal controller to the external console convey information, including continuous real-time telemetry of hydraulic pressure waveforms, system operating parameters, battery status, component temperature, and alarm information. This information is stored for later retrieval and analysis. The internal rechargeable battery, also placed abdominally, is lithium ion based and functions as an emergency or backup power source. It is continually recharged by external power received through the internal TET coil and can provide up to 20 minutes of operation while disconnected from the main power source. The internal TET coil receives high-frequency power that is transmitted across the skin from the external TET coil. The internal TET coil system electronics covert this oscillating current to a DC that is used to power the thoracic unit and to recharge the internal batteries.

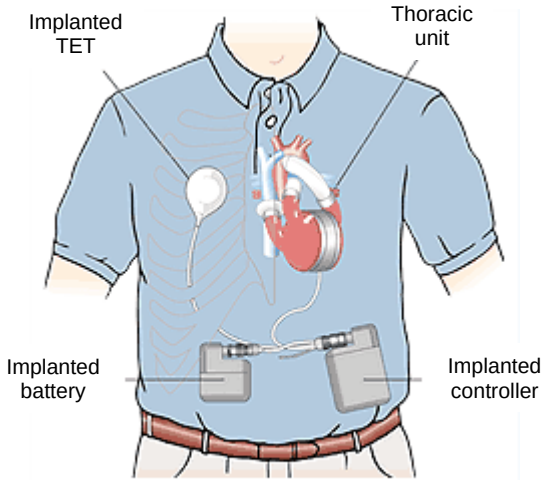

Figure 2.2 Internal components of the AbioCor™ TAH. Reprinted with permission from Abiomed, Inc.

The four external components consist of an external TET coil, batteries, a TET module, and a bedside console. The bedside console is used during implantation, recovery, and when the patient is in his/her primary residence. The bedside console provides clinicians with a graphic user interface for control and monitoring the implanted system through radiofrequency communication. The console can be configured to operate in different modes for implantation, recovery, and home monitoring. In addition, the console can be remotely monitored when connected to a telephone jack through a laptop computer. The rechargeable battery in the console allows to be disconnected from AC power for brief periods without discharging the patient's internal battery. When the patient is ambulatory, the external TET coil is connected to the portable TET module. The TET module delivers energy to the TET coil from the external batteries and contains basic alarms systems that are activated if there is

misalignment of the TET coil, low external battery voltage, or a general alarm indicating a potential problem with the system that is determined by re-establishing radiofrequency communication with the bedside console. The external batteries are lithium ion based and are able to provide up to one hour of support per pound battery allowing the patient to be completely free of the external power transmission for approximately four hours. The external batteries can either be carried in a vest or a handbag or attached to a Velcro belt. Technical data AbioCor™ TAH are: weight ~900 g, beat rate 75-150 beats per minute, flow rate 4-8 l/min, rotational speed of centrifugal pump 3000-10,000 rpm.
Abiomed, Inc. is also working on the next generation implantable prosthetic device, the AbioCor II. Incorporating technology both from Abiomed, Inc. and Pennsylvania State University (Hershey, PA, USA), the AbioCor II is smaller (35% reduction in size) and therefore able to fit significantly more of the adult population, and is being designed with a goal of five year reliability.
Initially, Abiomed, Inc. submitted the AbioCor™ TAH for marketing approval under the Humanitarian Device Exemption to the FDA in September 2004. Abiomed, Inc. intends to submit for an FDA Investigational Device Exemption in 2006 in order to begin clinical investigations with a purpose of seeking premarket approval by 2008. Currently, the AbioCor II is being implanted in animal studies.

ACcor TAH
The ACcor TAH of the Helmholtz-Institute for Biomedical Engineering, Aachen University of Technology (Germany), is being developed primarily for BTT and finally for use as a permanent heart replacement system. It consists of three main components: two diaphragms pump chambers, replacing the explanted ventricles functionally and anatomically, with inlet and outlet valves, and the electromechanical energy converter. The inlets of the pump chambers are connected to the natural atria while the outlets are connected to the aorta and the pulmonary artery, respectively. The energy converter consists of a brushless electronically commutated synchromotor and two reduction and hypocycloid gear units which transform the unidirectional rotational movement of the motor into translatory pusher plate excursions. Four acute animal tests in calves have been performed in cooperation with the university hospitals in Vienna (Austria) and Aachen (Germany). The ACcor TAH is capable of providing full circulation for 8.5 hours with a flow of 4-8 l/min.
A 20% smaller sized version of the ACcor TAH, the MiniACcor has been designed, manufactured and assembled. The MiniACcor pump unit is extensively tested within circulatory mock loops. The pump delivers flows between 4 to 7 l/min at aortic pressures of 80 to 140 mmHg at different pump rates.

2.1.1.2 Paediatric blood pumps
In early stages, hear failure in children is treated pharmacologically as in adults. As the disease severity increases, definitive therapy of heart failure in children consists of heart transplantation. Because sudden death in children awaiting heart transplantation is rare, the majority of deaths in this population are due to progressive heart and multi-organ failure and are therefore, at least in theory, amenable to salvage therapy with mechanical circulatory support [Rosenthal et al., 2000]. A important difference between the use of blood pumps in adults and children is the suitability of the devices. For adults, there are many choices of devices designed specifically for these patients, whereas for children, the choices are limited. There is substantially less experience with paediatric VADs, including extracorporeal membrane oxygenation (a technique best preserved for short-term support and often associated with a high rate of complications). Options for longer-term support are the Thoratec pneumatic VAD (Thoratec Corporation, Pleasanton, CA, USA) [Hill and Reinhartz,

2006; Reinhartz et al., 2001], the EXCOR® Pediatric (Berlin Heart AG, Berlin, Germany) [Etz et al., 2004], and the MEDOS/HIA System (MEDOS Medizintechnik AG, Stolberg, Germany) [Kaczmarek et al., 2005]. These devices are paracorporeal VAD systems employing pneumatically driven, thin membrane pumps to provide pulsatile flow and are available in a variety of pump sizes suitable for paediatric support. Nevertheless, paediatric mechanical support for children, infants, and neonates has started to attract more attention.

DeBakey VAD® Child
The DeBakey VAD® Child of MicroMed Technology, Inc. (Houston, TX, USA) is a miniaturised version of the DeBakey VAD® [Imamura et al., 2005; Morales et al., 2005]. The paediatric version employs the same axial flow pump used in the adult system with design modifications aimed at reducing the lateral space requirements for device implantation. These design modifications include a shortened inflow cannula with a more acute angle for the inflow tubing, a shortened plastic outflow graft protector, and reduced size of the flow probe on the outflow graft. Under the current Humanitarian Device Exemption programme of the FDA the DeBakey VAD® Child is used to provide temporary left ventricular support as a BTT for children from 5 to 16 years of age with a body surface area >0.7 m² and <1.5 m² and is designed to be implantable is this size range.

MVAD ventricular assist device
The MVAD of HeartWare Ltd (Sydney, NSW, Australia) is expected to serve as the basis for the development of a paediatric VAD. The size of the MVAD™ is approximately one third the size of the HVAD™, one of the smallest third generation pumps under development as a BTT. Minimally invasive techniques are used to implant the MVAD™ as intravascular device. Currently, the MVAD™ is available for animal studies as a prototype. Animal studies commenced in August 2005. The first human clinical investigations are expected within approximately two years.

PediaFlow™ VAD
The PediaFlow™ VAD is being developed by a consortium consisting of the University of Pittsburgh (Pittsburgh, PA, USA), Carnegie Mellon University (Pittsburgh, PA, USA), Children's Hospital of Pittsburgh, LauchPoint Technologies LLC (Goleta, CA, USA), and World Heart Corporation (Oakland, CA, USA) [Wearden et al., 2006; Wu et al., 2005]. The PediaFlow™ is based on a mixed flow pump featuring a magnetically levitated impeller capable of providing left, right, or biventricular support for children 3-15 kg in weight (birth to approximately two years). The miniature paediatric-sized heart pump has a size of about a quarter. The PediaFlow™ can be used for up to six months and is fully implantable with a percutaneous lead for powering the device. A prototype has been designed and built for an *in vivo* implantation in an ovine animal model.
Technical data: size 10×15 mm (height×diameter), rotational speed 13,000 rpm at a pressure of 100 mm Hg, flow rate 0.3-1.5 l/min.

PediPump™
The PediPump™ is under development at the Cleveland Clinic (Cleveland, OH, USA) specifically for children [Duncan et al., 2005]. The PediPump™ is a mixed-flow device based on a magnetic bearing pump design to provide support for the entire range of patient sizes encountered in paediatrics with a single pump. The enabling technology is drawn from an adult catheter pump resulting in a new impeller VAD suitable for supporting children from newborns to adolescents. The pump rotating assembly consists of an impeller in the front, front and rear radial magnetic bearings, and a motor magnet in its centre. Blood enters axially

at the inlet, and is turned in the impeller to exit the pump at an intermediate angle through the pump outside diameter. An inflow cannula is configured as appropriate for the size of the patient. Some arterial blood flows through windows at the rear of the pump under the influence of arterial pressure, washing and cooling the motor cap, before returning to the impeller. The rotor is supported on passive, radial magnetic bearings. Titanium shells seal all potentially corrodible components from blood and tissue. Unique features are the absence of a seal with suspension of the rotor on magnetic bearings resulting in high durability, and its small size (60×7 mm, length×diameter). Because of its small size, completely intravascular implantation may be possible for children beyond infancy. Furthermore, the device is suitable for left, right, and biventricular support. Animal testing was scheduled to commence in 2006.

Other devices
A paediatric VAD is under development at the University of Pittsburgh (Hershey, PA, USA) is a pulsatile flow device, based on the design of the adult-sized Pierce-Donachy VAD (Thoratec® #61650) and intended for BTT (expected maximum duration of use is six months). The infant-sized paediatric VAD has a dynamic stroke volume of approximately 13 ml. A larger 25 ml size for children is also planned. *In vitro* testing is being performed. The Pediatric Jarvik 2000 of Jarvik Heart, Inc. (New York, NY, USA) is an axial flow blood pump in a child and infant model version. Initial animal testing has commenced. Currently, a paediatric model control system and battery is being developed. The child and infant models will be implantable in any of the four chambers of the heart for chronic mechanical left, right or biventricular support.
Technical data child model: weight 17.8 g, length 5.5 mm, external volume 10 cm^3, rotational speed 10,000-14,000 rpm, flow rate 0.5-3 l/min. Technical data infant model: weight 12 g, length 3.8 mm, external volume 4 cm^3, rotational speed 16,000-24,000 rpm, flow rate 1.5 l/min.

2.1.2 Applications in the Netherlands
Ventricular assist devices (VADs) are in routine use as a bridge to transplantation (BTT), bridge to recovery (BTR), and long-term chronic support (LTCS). Also cardiac pacing and defibrillation devices are well established technologies. Currently, chronic animal experiments with the MiniACcor total artificial heart (TAH) are being planned at the Radboud University (Nijmegen, The Netherlands) in cooperation with the Heart- and Diabetes Centre in Bad Oeynhausen (Germany) and the clinic for Thoracic- and Heart Surgery in Nijmegen.

2.2 Cell/tissue-based approach for function recovery

Despite recent advances in medical and device therapy for heart failure, the incidence, hospitalization, and mortality rates continue to rise. The possibility of using cell-based therapies for people suffering an acute myocardial infarction, advanced coronary artery disease and chronic heart failure has made enormous advances in moving towards clinically applicable treatment options. Moreover, cell therapy of the heart seems to be the most abundantly practiced cell therapy in the clinic throughout the world.

2.2.1 State of development
Mechanism of action
There are several mechanism of action used, or claimed to be used, as a principle for the current cardiac cell therapy approaches. The classic idea is that delivery of the appropriate

stem cells would repair a damaged heart via active myocardial regeneration resulting from trans-differentiation of administered stem cells. Stem cells, regardless of origin, have the remarkable potential to develop into many different cell types in the body. When a stem cell divides, each new cell has the potential to either remain a stem cell or become another type of cell with a more specialized function, such as the beating cells of the heart. Stem cells may also stimulate heart repair through paracrine signalling actions [Gnecchi et al., 2005]. Stem cells are known to release angiogenic ligands, protect cardiomyocytes from apoptotic cell death, induce proliferation of endogenous cardiomyocytes, and may recruit resident cardiac stem cells. Furthermore, stem cells are known to stimulate neovascularisation [Kocher et al., 2001; Schuster et al., 2004] which also results in an enhancement of the self repair mechanism of the heart by allowing fast recruitment of systemically available stem cells and by preventing further cell death due to the new blood supply routes. Another proposed mechanism for trans-differentiation is the fusion of exogenous stem cells with host cardiomyocytes which lead to cardiac repair [Nygren et al., 2004]. Finally, it is suggested that the effects of stem cells are mediated by altering mechanical properties to strengthen a myocardial infarction scar, thereby preventing deterioration in cardiac function [Fujii et al., 2003]. Most probably, a combination of these mechanisms is involved in most cell therapy approaches.

Cell source

The cell source of the applied cells is one of the major variables among the different cardiac cell therapies. One of the most promising and also controversial cell sources are embryonic stem cells, which are totipotent cells (able to differentiate in many cell types) derived from the inner cell mass of blastocysts. In theory, infinite numbers of cardiomyocytes could be obtained from human embryonic stem cell clones. The use of embryonic stem cells *in vitro* differentiated to cardiomyocytes has shown to improve cardiac function in several rodent models [Behfar et al., 2002; Klug et al., 1996; Min et al., 2002; Yang et al., 2002].

The use of resident adult cardiac stem cells is thought to represent a therapeutic target that, if enhanced, could induce cardiac self-repair by mediating mechanisms of repair and replacement. Intriguingly, cardiac stem cells can be clonally expanded from human myocardial biopsies [Messina et al., 2004]. An easily accessible source of cardiac stem cells is the auricle of the heart. This appendix of the heart contains many stem cells and can easily be missed since it has no direct role in the primary function of the heart.

Autologous skeletal myoblasts or satellite cells are another potential source used for cardiac repair. These cells are the reservoir of regenerative cells for skeletal muscle tissue and have the ability for self renewal and differentiation in case of muscle injury [Siminiak et al., 2004]. These cells normally lie in a quiescent state under the basal membrane of mature muscular fibres. Myoblasts can be isolated from skeletal muscle biopsies and expanded in vitro. Because they are easily obtainable and capable of regeneration, researchers are attempting to use myoblasts or satellite cells as a source of cells to repair the heart.

Bone marrow mononuclear cells are used since these cells have been demonstrated to home to infarcted myocardium after reinfusion [Strauer et al., 2002]. These cells include hematopoietic stem cells that are involved in the process of neovascularisation and mesenchymal stem cells that have the potential to differentiate into many cell types including cardiomyocyte-like cells. In many cases, mesenchymal stem cells are isolated from the bone marrow and culture expanded before they are applied to the patient.

Endothelial progenitor cells are used for the strategy that focuses on prevention of apoptosis. These cells are present in the adult bone marrow and stimulate neovascularisation. New blood vessel ingrowth can prevent cell death and favours remodelling of the heart tissue which leads to some degree of cardiomyocyte regeneration. Finally, umbilical cord blood containing

both hematopoietic stem cells and mesenchymal precursor cells have been reported to be used for cardiac cell therapy approaches.
Besides the type of cells also the use of autologous versus allogeneic cells is an important variable between the different available approaches. Whereas autologous cell-based therapy poses no risk of rejection, an 'off the shelf' allogeneic cell product would be much more cost effective and much easier to administer and could potentially allow delivery of greater numbers as compared to an autologous cell therapy approach. An exception is mesenchymal stem cells, which appear to avoid the problem of rejection by being hypoimmunogenic. These cells lack MHC-II and B-7 co-stimulatory molecule expression, thereby preventing T-cell responses, and induce a suppressive local microenvironment through the production of prostaglandins and other soluble mediators [Ryan et al., 2005; Zimmet and Hare, 2005]. As such, mesenchymal stem cells may allow allogeneic cell therapy while avoiding rejection.

Cell delivery
In general, the cells to be applied can be delivered in three ways: I) Direct injection into the hart, II) Infusion into the blood stream also referred to as transvascular approaches, and III) Delivered as 3-dimensional patches.

I) Direct injection into the hart
Direct injection is often based on the delivery of cells that are aimed to function as cardiomyocytes (e.g., mesenchymal stem cells or myoblasts/satellite cells). The cells can either be injected into the target area by open heart surgery or via a catheter-based approach, such as via the coronary artery supplying an infracted zone or across the aortic valve in the endocardial surface. A catheter-based delivery is less invasive and minimises the recovery period of the patient. This local administration seems to be the most effective route of delivery and is also the preferred route for cell delivery in patients with strongly occluded arteries that precludes transvascular cell delivery or when cell homing signals are expressed at low levels in the heart (in scar tissue). However, injected cells into ischaemic or scarred myocardium are reported to create islands of cells with limited blood supply and may lead to poor cell survival [Bel et al., 2003].

II) Transvascular approaches
Transvascular strategies are especially suited for the treatment of recently infarcted and reperfused myocardium when chemo-attractants and cell adhesion molecules are highly expressed. Infusion can be performed either through the coronary artery or through intravenous infusion. Regarding intracoronary infusion, a transient balloon can be inflated in order to stop the local blood flow and maximise the contact time of the cells with the microcirculation of the infarct-related artery [Wollert and Drexler, 2005]. Concerning intravenous infusion, cells are completely dependent on the homing capacity of the cells. However, the cells are also reported to home to other non-cardiac organs and the clinical applicability appears to be suboptimal [Hofmann et al., 2005].

III) 3-dimensional patches
The last group comprises the use of 3-dimensional cultured patches. These can be created by combining cultured cells with substrate materials, which offers the advantage that the scaffold materials can be shaped in any 3-dimensional form on both a macroscopic and microscopic level. One of the leading research groups that are developing such an approach is the group of Zimmermann at the University Medical Center Hamburg-Eppendorf in Germany. They have created engineered heart tissue by using neonatal rat heart cells, which is a mixed population of heart cells including cardiac myocytes, fibroblasts, smooth muscle

cells, endothelial cells and macrophages. The cells are combined with collagen I and Matrigel, reconstituted in circular molds and subjected to mechanical strain. Under these conditions, cardiac organoids developed spontaneously and show contractile as well as electrophysiological properties of working myocardium. This results in large (thickness/diameter, 1-4 mm/15 mm), force-generating engineered heart tissue. This engineered heart tissue has demonstrated to form thick cardiac muscle layers when implanted on myocardial infarcts in immune-suppressed rats. When evaluated 28 days later, engineered heart tissue showed undelayed electrical coupling to the native myocardium without evidence of arrhythmia induction. Moreover, engineered heart tissue prevented further dilation, induced systolic wall thickening of infarcted myocardial segments and improved fractional area shortening of infarcted hearts compared to controls (sham operation and noncontractile constructs). Thus, this study demonstrates that large contractile cardiac tissue grafts can be constructed in vitro, can survive after implantation and can support contractile function of infarcted hearts [Zimmermann et al., 2006].
A similar approach is developed at the Massachusetts Institute of Technology (MIT). They have been able to force heart cells isolated from newborn rats to mature into cardiac muscle by placing them on support scaffolds of biocompatible materials and exposing them to electrical stimulation and a relatively high oxygen concentration [Radisic et al., 2006]. Under these conditions, the heart cells fuse into functional tissue and begin beating synchronously. The potential future production of contractile patches for human use is illustrated in Figure 2.3. A biodegradable polymer invented at MIT, called biorubber, is pierced by using a laser to pierce to create a fine network of channels. Each piece is a rectangle roughly 1 cm^2 in area and up to 3 mm thick. Subsequently, the biorubber is seeded with the three major cell types of a heart: cardiomyocytes, endothelial cells, and fibroblasts (e.g. by using adult stem cells derived from fat tissue). As the cardiomyocytes beat, they adhere to and tug on one another, helping them to communicate electrically and to secrete the growth hormones they need to survive. To be strong enough to replace dead heart tissue in people who have had heart attacks, the contractile patches must be at least 5 mm thick. However, currently the patches reach only a thickness of approximately 1-2 mm.

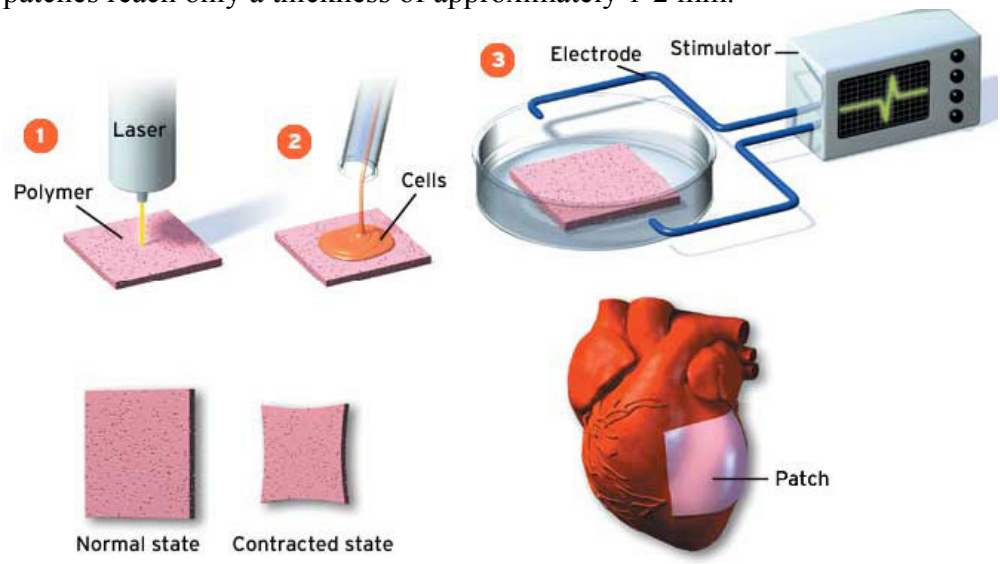

Figure 2.3 Engineered heart patches as in development at the Massachusetts Institute of Technology. 1) A thin square of biodegradable polymer is perforated with a laser to form a fine network of channels. 2) The polymer patch is seeded with the three types of heart cells. 3) The patch is stimulated with electric current. 4) When mature, the patch beats like a piece of living heart. 5) Once a way is found to increase the thickness of the experimental patches, they will be strong enough to be grafted onto human hearts (Reproduced with permission of Bryan Christie Design).

A further hurdle to overcome is the generation of a vascular network within the cultivated tissue and integrate it with the host tissue's own vascular network. Furthermore, the transplanted patch must respond to the host heart's electrical stimulation by synchronous contractions.
Another fascinating approach has been proposed by Shimizu et al. [2003], who use materials to create electrically communicating 3-dimensional cardiac tissue layers. They have reported to adhere cells on tissue-culture plates previously coated with poly(N-isopropylacrylamide) (PIPAAm), a temperature-sensitive polymer. At 37 °C PIPAAm is hydrophobic, enabling cell adhesion and access to the binding sites offered on this modified surface. At a lower temperature such as 32 °C, the surface becomes hydrophilic and inappropriate for cell adhesion due to the rapid hydration and swelling of PIPAAm.
Using polyvinylidene difluoride (PVDF) membranes, which are hydrophobic, the detaching cell layers can be collected and handled, providing up to four conducting layers of synchronously beating cardiomyocytes. After implantation of these patches in rats with induced myocardial infarction, an improved myocardial contractility was observed. Furthermore, a vascular network in the transplanted area appeared within a few days after implantation.

Clinical studies
Many hospital, universities and companies are involved in the field of heart cell therapy. An abundance of preclinical data demonstrates safety, feasibility and efficacy, justifying the current entry into clinical trials of heart cell therapy in humans. This position, however, is controversial, with some arguing that trials are premature because mechanistic insights are insufficiently addressed [Chien, 2004]. However, conducted rigorous clinical trials are also seen as an essential component in the process of understanding the scientific underpinnings of cardiac regeneration and its therapeutic utilisation. At present, many early clinical studies have been conducted worldwide.
To our knowledge no human clinical studies have been initiated using embryonic stem cells because of both the ethical issues surrounding access to embryos and the possibility of teratoma formation, suggested by a study injecting embryonic stem cells in skeletal muscle [Thomson et al., 1998]. Neither have clinical trials been reported using human adult cardiac stem cells and umbilical cord blood stem cells. Regarding skeletal myoblasts and satellite cells, a growing body of experimental data and initial clinical studies has shown not only engraftment of donor cells but also improvement in global cardiac pump function due to enhanced viability in the injected myocardial areas [Hagege et al., 2003; Murry et al., 1996; Taylor et al., 1998]. The publication of Menasche et al. [2001] describing the first patients to receive skeletal myoblasts spawned a profusion of small clinical studies investigating cellular therapy for cardiac repair. This approach was further developed by Genzyme / Biotherapeutics / Myosix and has been evaluated in a phase II clinical trial [Menasche et al., 2003]. This trial was designed to determine whether cell therapy with autologous skeletal myoblasts can replace damaged myocardium cells and restore muscle function following a heart attack, or to safely halt a patient's further progression of ischaemic heart failure. Investigators in this Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial harvest patients own skeletal myoblast cells (autologous) prior to bypass surgery through a small biopsy in the leg. These cells are multiplied in the laboratory over approximately 21 days using a proprietary cell-culture technique. The investigators then inject the cells into the damaged region of a patient's heart during a coronary artery bypass operation. This multi-centre clinical trial has been designed to assess the safety and efficacy of two doses of autologous skeletal myoblasts, as compared to placebo, in the treatment of ischaemic heart

failure. The MAGIC trial is conducted in Europe, including centres in Belgium, Denmark, France, Germany, Italy, and the UK.

The use of bone marrow (mono)nuclear cells is another widely studied cell-based therapy for human applications. Altogether, many patients have been tested, and the totality of evidence from these studies supports safety of the tested cell therapy. Nevertheless, in general no significant effect has been demonstrated by these unselected bone marrow nuclear cells. The therapeutic effect of mesenchymal stem cells has been studied in very limited studies and no conclusions can yet be made. Overall, a flurry of small, mostly uncontrolled clinical studies exploring the safety and feasibility of stem cell therapy has been conducted. These studies have used a myriad of different cell types and preparations, each in a small number of patients with different disease states. In the aggregate, this preliminary clinical evidence suggests that stem cell therapy might work.

The field is now rapidly moving to large, randomised, placebo-controlled, double-blind studies with clinical end points to gather more safety and efficacy data. These studies should be performed in specified subgroups, which should include patients with acute myocardial infarction and heart failure since these groups have been excluded in most trials for safety concerns. For each of these subgroups and proposed cell therapies the following should be determined: optimal cell number required to gain maximal clinical effect, the optimal *ex vivo* manipulation of the cells, the most effective delivery system of the cells and information concerning the mechanism of action. Also the effect of possible concomitant medication should be integrated in the future clinical studies. After these studies the cell therapy treatments could be upgraded to standard treatments, whereas also the first market applications might be expected in the coming years.

2.2.2 Applications in the Netherlands

The Interuniversitair Cardiologisch Instituut Nederland (ICIN) is an alliance of the 8 university cardiology departments in the Netherlands that stimulate, co-ordinate and execute scientific research in the field of cardiovascular diseases (Figure 2.4).

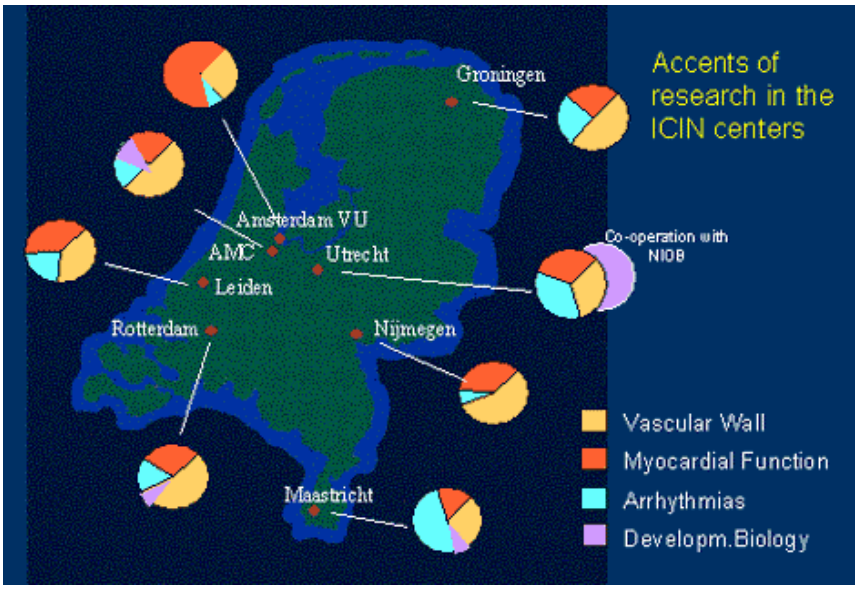

Figure 2.4 Overview of the Interuniversitair Cardiologisch Instituut Nederland (ICIN) *[www.icin.nl]*.

The ICIN also seeks co-operation with non-academic hospitals, institutions of basic science, or fellow institutes of the Royal Academy. For clinical studies organised by the ICIN, patients are basically recruited in the participating hospitals. Nevertheless, the larger non-academic hospitals in the Netherlands are often invited to join the project. In March 2005, ten large hospitals have started a clinical trial (HEBE trial, named after the Greek goddess of eternal youth), which is a cooperation between the ICIN and the Dutch Heart Foundation (Nederlandse Hartstichting). The HEBE trial is a multi-centre, prospective, randomised, 3-arm open trial with blinded evaluation of end points. Patients with acute large myocardial infarction treated with primary percutaneous coronary intervention (PCI) will undergo magnetic resonance imaging (MRI) and echocardiography. A total of 200 patients are randomised to treatment with (1) intracoronary infusion of autologous mononuclear bone marrow cells, (2) intracoronary infusion of peripheral mononuclear blood cells, or (3) standard therapy. Mononuclear cells are isolated from bone marrow aspirate or venous blood by density gradient centrifugation. Within 7 days after PCI and within 24 hours after bone marrow aspiration or blood collection, a catheterisation for intracoronary infusion of the mononuclear cells in the infarct-related artery is performed. In all patients, follow-up will be obtained at 1, 4, and 12 months. MRI and catheterisation are repeated at 4 months, and all images are analysed by a core laboratory blinded to establish randomisation. The primary end point of the study is the change in regional myocardial function in dysfunctional segments at 4 months relative to baseline, based on segmental analysis as measured by MRI [Hirsch et al., 2006]. Unfortunately, no results have been published yet.

In the Erasmus Medical Centre in Rotterdam, Dr. Peter Smits is using autologous skeletal myoblasts as a cell source for heart cell therapy. This cell therapy (MyoCell) is developed by the company Bioheart, Inc. (Sunrise, FL, USA) and is intended to be marketed as a human medicinal product. MyoCell is a tissue regeneration therapy product for the treatment of a continuum of cardiovascular disease states ranging from heart attack to end-stage heart disease. For the MyoCell product, autologous myoblasts are isolated from five to ten grams of skeletal muscle and subjected to an enzyme disassociation process that selects a very specific sub-population of myoblasts. The cells are cultured in proprietary media at a Bioheart controlled cell culturing facility, shipped to the cardiologist, and implanted into the damaged area of the myocardium. The myoblasts are injected either via a percutaneous catheter system or as part of a coronary artery bypass grafting surgery. Furthermore, the used injected media keeps the cells quiescent for up to eight days, which should allow the muscle to build up gradually instead of suddenly and result in better muscle formation. Ultimately, the product should be able to engraft, proliferate, adapt to the cardiac microenvironment and support cardiac workload.

In a pilot safety and feasibility study in Rotterdam, five patients with symptomatic heart failure after an anterior wall infarction have been treated with this product [Smits et al., 2003]. After a culturing process, 296 +/- 199 million cells were harvested and 196 +/- 105 million cells were transendocardially injected into the infarcted area with the catheter system (Biosense-Webster, Waterloo, Belgium). The results of this pilot study have demonstrated the potential and feasibility of percutaneous skeletal myoblast delivery as a stand-alone procedure for myocardial repair in patients with post-infarction heart failure. However, more data are needed to confirm its safety and efficacy. Although more patients are being treated by using this cell therapy, no further results have yet been published to our knowledge.

A clear difference between this approach and the approach used with the HEBE trial is the mechanism of action. The mechanism of bone marrow derived stem cells is probably mainly related to induction and stimulation of angiogenesis, whereas skeletal myoblasts are more likely to contribute to recovery of left ventricular function by the direct engraftment of contractile cells. Furthermore, the use of skeletal myoblasts is known to be correlated with

arrhythmias. Whereas stem cells obtained from the bone marrow contain specific communication proteins needed for the transfer of the electric signals generated by the heart, skeletal myoblasts do not contain these specific proteins. Electrical signals that normally flash through the heart in order to accomplish an organised contraction are then blocked and the signal is forced to make a detour that results in arrhythmias. Also during the clinical trials performed by Dr Smits, several deaths due to arrhythmias have been reported. Nevertheless, it remains difficult to pinpoint the actual cause of these observed arrhythmias, since the patient group is highly susceptible for arrhythmias [Smits, 2004].
Also clinical trials at the Leiden University Medical Centre have been reported in which bone marrow cells have been used [Beeres et al., 2006a; Beeres et al., 2006b]. In 25 patients (mean age 64 +/- 10 years, 21 men) with refractory angina, a total of 84 +/- 29 x 10^6 bone marrow-derived mononuclear cells was injected intra-myocardially in ischaemic regions. Anginal symptoms and quality of life were evaluated at baseline and at 3, 6, and 12 months. Gated single-photon emission computed tomography was performed at baseline and at 3 and 12 months to assess myocardial perfusion and left ventricular function. Although one patient died of intracranial haemorrhage, the results revealed that the heart function (based on the Canadian Cardiovascular Society Class) improved, the quality of life improved, the number of segments with ischemia per patient decreased and the left ventricular ejection fraction and regional wall motion were demonstrated to be increased due to the cell therapy. Nevertheless, the amount of patients is insufficient to draw any final conclusions.
In April 2006, a clinical trial was forced to be cancelled by the Dutch Central Committee on Research Involving Human Subjects (CCMO) since the trial had been initiated without approval of the CCMO. The manipulation of the stem cells before implantation was performed by the Dutch company Cells4Health BV (Leuvenheim, The Netherlands). Cells4Health BV is a specialised company for the harvesting, treatment and storage of stem cells derived from both umbilical cord blood and bone marrow. Currently, the company is still offering a treatment of acute myocardial infarction called Health-Cardiac MI. This treatment is performed in collaboration with the University Hospital in Gaziantep in Turkey. As stated on the webpage of Cells4Health, the first patient (candidate for heart transplantation) has already been treated. This offered treatment is however not acknowledged by the Dutch National Health Service and is therefore not covered by the health insurers and can not be performed in the Netherlands.

2.3 Conclusion

Numerous medical devices have been developed for mechanical circulatory support in patients with end-stage heart failure. Ventricular assist devices (VADs) are in routine use as a bridge to transplantation (BTT), bridge to recovery (BTR), and long-term chronic support (LTCS). The latest generation of VADs includes axial and centrifugal flow blood pumps. Also cardiac pacing and defibrillation devices are well established technologies. Currently, one total artificial heart (TAH, i.e. AbioCor™) is being used under the Humanitarian Device Exemption in the USA. In addition, the next generation TAH (i.e. AbioCor II) is being developed, which is smaller in size and therefore suitable for more patients. In Europe, the ACcor TAH and the MiniACcor TAH are currently being developed. These devices have been tested in animal studies and circulatory mock loops. In the Netherlands, chronic animal experiments with the MiniACcor are being planned at the Radboud University Nijmegen in cooperation with the Heart- and Diabetes Centre in Bad Oeynhausen (Germany) and the clinic for Thoracic- and Heart Surgery in Nijmegen.

Cell therapy of the heart seems to be the most abundantly practiced cell therapy in the clinic. Several approaches regarding cell source and cell delivery are being evaluated, of which the infusion and injection of bone marrow derived stem cells are the most abundant. This also accounts for the clinical studies that are performed in the hospitals in the Netherlands. Many studies are ongoing and more are expected to be initiated in the future. In particular, a large multicentre trial in ten large Dutch hospitals is going on, as well as separate trials in Rotterdam and Leiden. In 2006, a clinical trial at the Medical Centre of Twente was cancelled by the Dutch Central Committee on Research Involving Human Subjects (CCMO) after 8 of 10 patients had been treated with injected stem cells. The trial had been initiated without approval of the CCMO. The manipulation of the stem cells before implantation was performed by the Dutch company Cells4Health BV (Leuvenheim, the Netherlands), a specialised company for the harvesting, treatment and storage of stem cells derived from both umbilical cord blood and bone marrow. Currently, the company is still offering a treatment of acute myocardial infarction called Health-Cardiac MI. This treatment is performed in collaboration with the University Hospital in Gaziantep in Turkey. As stated on the webpage of Cells4Health, the first patient (candidate for heart transplantation) has already been treated. This treatment is not acknowledged by the Dutch National Health Service and is therefore not covered by the health insurers and can not be performed in The Netherlands.
Unfortunately, no major conclusions can yet be drawn based on the published studies conducted in the Netherlands. Based on the results obtained worldwide, feasibility and relative safety of cardiac cell therapy has been demonstrated. However, also large variation in efficacy is obtained which ranges from negligible to marginal. Although it can be considered that the proof-of-concept has been demonstrated, there is a need to explore and clarify the mechanism of action in order to improve efficacy. It can therefore be expected that many clinical studies as well as non-clinical studies will remain to be conducted in the coming years throughout the world, including the Netherlands.

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3. Lungs

The natural lung represents a remarkable organ for gas exchange. The lung's principal function is to transport oxygen from the atmosphere into the bloodstream, and to excrete carbon dioxide from the bloodstream into the atmosphere. This exchange of gases is accomplished in the mosaic of specialized cells that form millions of tiny, exceptionally thin-walled air sacs called alveoli. The alveoli of the lung, the tiny gas sacs at the termini of all the branching airways of the lung, offer intimate contact between inspired gas and blood flowing through capillaries in the lung. The O_2 and CO_2 diffusing capacities of the lungs are proportional to the gas exchange area of the alveolar-capillary membrane and to the inverse of the diffusion distance across the alveolar-capillary membrane into blood. The substantial gas exchange capacity of the lung stems from an alveolar-capillary area comparable to a tennis court surface, 100–150 m^2, packaged compactly with a high surface area to blood volume ratio of approximately 300 cm^{-1} and a diffusion distance between gas and blood phases of no more than about 1 μm. The natural lung can provide gas exchange ranging from resting levels for both O_2 and CO_2 (about 200–250 ml/min in average adults) to 10–20 times that under exercise conditions, and it does so using room air as its oxygen supply gas. Moreover, the lungs have a variety of functions other than gas exchange, including several metabolic functions. These functions include producing, storing and inactivating various vasoactive and coagulation modulating molecules [Zwischenberger, 2001]. The native pulmonary bed also has the property to act as a filter to prevent clot and other debris from entering the systemic circulation causing stroke. In addition to respiratory functions the lungs also:

- influence the concentration of biologically active substances and drugs used in medicine in arterial blood
- regulate the hydrogen ion concentration in the blood
- serve as a physical layer of soft, shock-absorbent protection for the heart, which the lungs flank and nearly enclose.

[Wikipedia; http://en.wikipedia.org/wiki/Lung]
The most important diseases of the lung which may necessitate organ transplantation or artificial organ support are cancer, acute respiratory distress syndrome (ARDS) and cystic fibrosis.

3.1 Medical device-based approach for function recovery

For patients with most severe acute respiratory distress syndrome (ARDS) conservative treatment with lung protective ventilation is often not sufficient to prevent life-threatening hypoxemia and additional strategies are necessary. While adequate gas exchange may be achieved, ventilation may cause barotraumas, volutrauma, oxygen toxicity complications and possibly worsen the patient's condition. While conservative therapy was optimized using lung protective ventilation with permissive hypercapnia, positive end-expiratory pressure, spontaneous breathing during pressure controlled ventilation, prone position and hydrocortisone therapy in stress doses, mortality remained about 25-40%. An artificial lung could serve as a rescue and/or supplement to ventilators, reducing the need for an aggressive ventilation protocol, thus reducing the complications from ventilation management during the treatment of acute respiratory failure or bridge to lung transplant.

Artificial lungs are medical devices designed to take over or supplement the respiratory function of the lung: oxygenating the blood and removing carbon dioxide. They are generally classified as extracorporeal, paracorporeal, intravascular, or intrathoracic devices (see figure 3.1). They are aimed to provide the following benefits over mechanical ventilation:

- Elimination of sedation allows the patient to stay alert, eat and communicate
- Elimination of ventilator associated pneumonia eliminates dangerous complications, and should reduce cost of care and length of stay in the ICU.
- Avoidance of intubation allows the patient to eat, speak and prevents tracheal injury and sinus infection.
- Reduction in weaning failure (from ventilator support) should reduce length of stay in the ICU and potential mortality.
- Reduction in tracheostomies will reduce an invasive surgical procedure to the larynx
- Reduced lung injury may reduce the incidence of death.

[http://www.alung.com/benefits.html]

The artificial lungs used clinically today are mainly extracorporeal membrane blood oxygenators (ECMO), primarily used in operations requiring cardiopulmonary bypass, but also used less frequently for support of patients with respiratory failure. The ECMO can provide total gas exchange for weeks resulting in recovery from severe respiratory failure in 40% of adult patients. ECMO has also been used successfully for treatment of lung transplant rejection or edema. Although occasionally successful as a bridge to transplant, ECMO requires multiple blood transfusions, is complex, labor intensive, time limited, costly, non-ambulatory, and prone to infection. The ECMO circuit, that includes pumps, reservoirs, heat exchangers and long lines of tubing presents a high priming volume and entails a risk of haemolysis, clotting and plasma leakage due to the blood contact surface.
The growing incidence of lung disease associated with the ageing population has encouraged work toward next generation artificial lungs that may be used to successfully treat patients with a variety of respiratory failures. Next generation artificial lungs include:

- intravascular approaches (respiratory catheters placed within the vena cava)
- paracorporeal approaches (wearable devices that will be attached directly to patients)
- intrathoracic approaches (devices placed within the thoracic or abdominal cavities).

Intravascular artificial lungs are aimed primarily at treatment of acute respiratory failures, whereas paracorporeal and intrathoracic artificial lungs are aimed primarily at bridge-to-transplant respiratory support because of the more invasive procedures required to implement them.

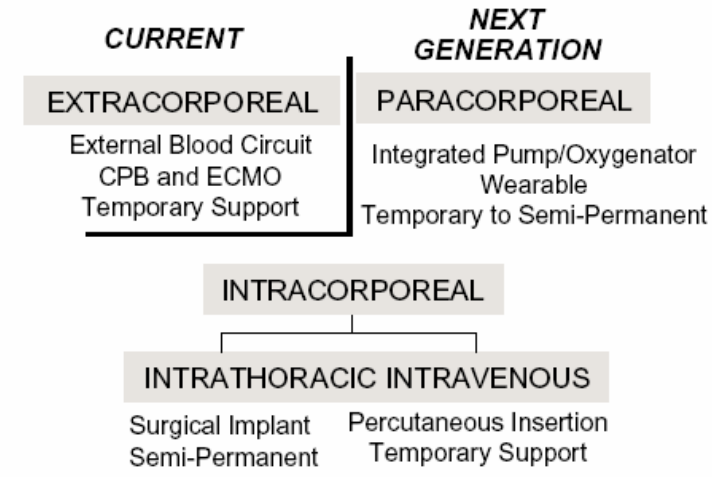

Figure 3.1 Towards the implantable artificial lung *(Source: Federspiel and Svitek, 2004)*

3.1.1 State of development

Intravascular oxygenator

Intravascular artificial lungs have been studied and are being developed as a less expensive, less personnel intensive alternative to respiratory support with traditional extracorporeal artificial lungs. Anatomical and physiological constraints of device placement in major blood vessels of the human body impose significant challenges in developing intravascular artificial lungs. Most of the intravascular devices that have been developed are intended for insertion through a peripheral vein (femoral or jugular) and placement in the vena cava, the largest blood vessel in the body through which blood returns to the heart. The adult human inferior vena cava ranges on average from 2.2 cm to 3.3 cm in diameter and the superior vena cava ranges from 1.5 cm to 2.2 cm. Intravascular artificial lungs must be compact for insertion, yet possess sufficient membrane area to achieve adequate respiratory support. The primary objective of intravascular artificial lungs is to supplement the gas exchange of a failing lung, but not completely replace it. Respiratory support at 40–60% of the body's resting metabolic needs has generally been considered an appropriate target for intravascular artificial lungs.

Intravascular oxygenation (IVOX)

The concept of intravascular oxygenation (IVOX) was introduced by Mortensen and Berry [1989], who investigated the possibility of achieving gas exchange by introducing a bundle of hollow fibers into a blood vessel. The early IVOX was designed to be inserted through the femoral or the jugular vein and to occupy the vena cava for gas exchange. The fibers were made from polypropylene, surrounded by an ultra-thin siloxane coating that acted as a membrane permeable to oxygen and carbon dioxide, but not plasma. Another covalently bonded heparin derivative coating was used to increase thrombo-resistance of the device. An important property of the IVOX involved crimping of the hollow fiber membranes along its length to present an opportunity for secondary currents to flow around the fibers. This prevented the device from depending on the passive flows passing the fibers to initiate gas exchange. Compared with extracorporal oxygenation IVOX presents a smaller blood contact surface, reduces the size of the insertion, reduces the risk of infection and surgical operation time, and sets the priming volume to zero. No blood tubes and heat exchangers are required in the system. The clinical trials on Mortensen's IVOX system demonstrated a rather low oxygen transfer, corresponding to only 20 - 30% of the whole body requirement and proved to have no effect on the mortality in ARDS patients. This low transfer rate is ascribed to blood flowing parallel to the fibers, causing an increased boundary layer thickness that hinders gas exchange. Enhanced gas transfer is generally through cross-flow and blood mixing. Moreover, parallel flow causes stagnation and increases the risk of thrombosis. Two modified systems to improve the blood flow around the fibers are under development; the Hattler catheter and HIMOX.

Hattler catheter

The Hattler catheter uses technology similar to Mortensen's IVOX system (see Figure 3.2). It is also an intravascular gas exchange device implanted into the vena cava or right atrium, but the ± 800 hollow fibers that are woven into a fabric, surround a small pulsatile balloon. The balloon is rapidly pulsating at a rate of up to 300 'beats' per minute, creating convective currents around the hollow fibers which allows for enhanced oxygenation of the blood and removal of carbon dioxide. In this way about 50% of the patient's oxygen needs can be provided by the oxygenator. The Hattler catheter is intended to assist temporarily the function of the lungs, giving them time to heal. It could meet a need for patients with acute respiratory failure, such as those with emphysema, or those who have suffered trauma or infections to the lungs. In this way theu can provide an alternative to the current standard of care, which is the

use of ECMOs that can cause life-threatening complications and death in more than half of those who are treated with them.
[http://www.mirm.pitt.edu/programs/medical_devices/lung.htm]
The Hattler catheter is still under development. An email communication with Alung Technologies Inc. learned that the device is still at a pre-clinical stage.

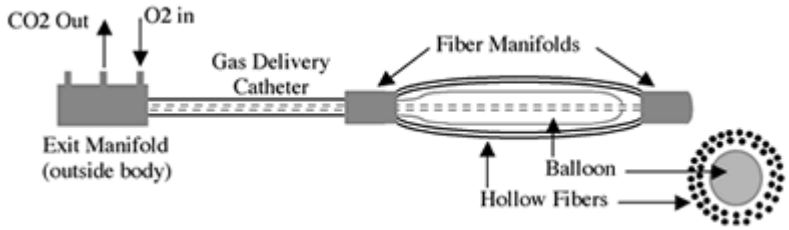

Figure 3.2 Hattler catheter, including pulsatile balloon. *(Source* http://www.alung.com*, courtesy dr. W.J. Federspiel)*

Highly integrated intravascular membrane oxygenator (HIMOX)
Researchers from the Department of Cardiovascular Enginering of the Helmholtz-Institute for Biomechanical Engineering at the RWTH in Aachen, Germany are working on a improved IVOX device, which they named the highly integrated intravascular membrane oxygenator (HIMOX). This device has improved fiber configuration for better flow and gas exchange properties so that the efficiency is independent from the anatomical and fluid dynamic conditions in the venous system (see Figure 3.3).
Core of the HIMOX are several serially connected tubular shaped hollow fiber bundles. The bundles are mounted on a central catheter, which guides them during insertion through the vena femoralis into the vena cava and serves as pipeline for gas removal. Since each bundle is fixed at both ends to a slide, which is free to glide on the catheter, bundles can assume two major configurations. During the insertion through the femoral vein, the fibers are arranged parallel to the catheter, much like in the original IVOX device. Once the vena cava is reached, the bundles are compressed and twisted by means of a tool connected to the catheter outside the body. In the expanded configuration, the bundles are shaped like very thin discs, which are perpendicular to the axis of the catheter and present a tight, spiral and homogenous fiber configuration, enhancing blood mixing and gas exchange through convective processes. However, high fiber density presents a detrimental side effect as it increases the blood pressure drop across the fibers. For this reason a micro axial pump is integrated in the HIMOX [Cattaneo et al., 2004, 2005, 2006].
[http://www.ukaachen.de/go/show?ID=4377566&DV=0&COMP=download&NAVID=1792692&NAVDV=0]
Both the university's website and the publications by Catteneo et al. [2004, 2005, 2006] show that the HIMOX is in an early stage of development. The enhanced gas exchange by twisting and compressing the fiber bundle in the vena cava has been demonstrated in vitro for a single bundle. The pressure drop over the single bundle is already high, which raises the question whether a single micro blood pump will be able to compensate. Technical issues like the controlled twisting and compressing of the fiber bundles in the vena cava have not been addressed jet. Also micro axial blood pump is still under development by the university. It is unlikely that the HIMOX will be available for clinical testing with a few years.

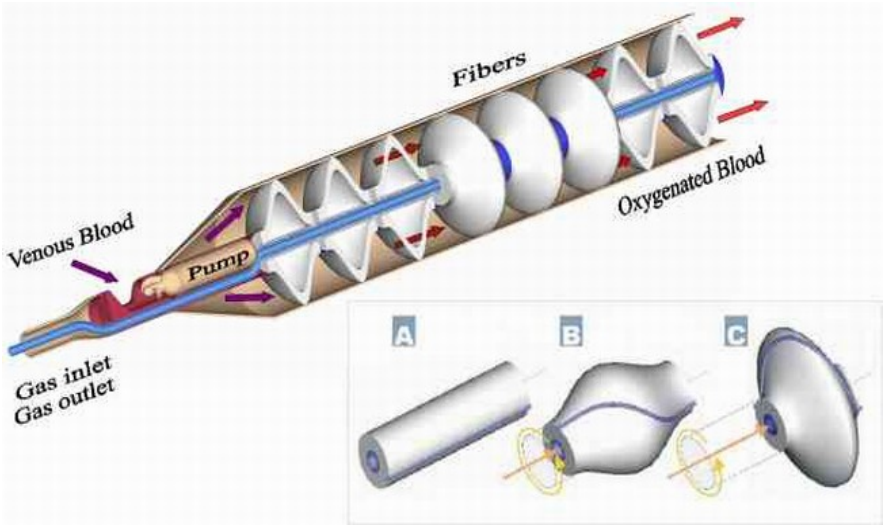

Figure 3.3 Construction of HIMOX *(Source: Cattaneo 2004, courtesy SAGE Publications Ltd)*

Pumpless extracorporal lung assist (pECLA)

With the extra corporeal membrane oxygenators that are used in heart-lung machines a blood pump is necessary to overcome the flow resistance of cannulae and oxygenators and to achieve sufficient blood flow. Using newly designed oxygenators with reduced pressure drop, the difference between arterial and venous blood pressure is sufficient to achieve adequate extracorporeal blood flow.

The heart pumps blood through the pumpless lung assist device via a femo-femoral shunt created by percutaneous arterial and venous cannulation with high-flow cannulae. The low impedance of the lung assist device avoids the use of an artificial blood pump. Due to this principle, adequate mean arterial pressure is mandatory and low cardiac output or cardiogenic shock are contraindications for interventional lung assist [Liebold, 2002] This type of device is attached to the systemic circulation and receives only part of the cardiac output for extracorporeal gas exchange. This allows complete CO_2 removal, which can be controlled by varying sweep gas flow. Oxygenation depends on shunt, arterial oxygen saturation and other variables.

Advantages of an interventional lung assist (ILA) are avoidance of all pump related complications, reduced blood contacting surfaces and simplified clinical management. Disadvantages are the indirect control of blood flow which is the result of the arterio-venous pressure gradient, the low oxygen transfer capacity since arterial already oxygenated blood is flowing into the device, the arterial cannulation which might impose local problems to the cannulated vessel and distal blood flow, and the arterio-venous shunt perfusion up to 25% of cardiac output which needs to be achieved by the left ventricle. Indications for ILA are not formally established in well controlled clinical trials but patients with severe acute respiratory failure and severe hypercapnia seem to benefit best. In case of severe hypoxemia oxygen transfer rate is often not sufficient and veno-venous ECMO is necessary. ILA enables a more protective ventilation strategy with a pressure controlled or pressure support ventilation mode with reduced airway pressure and tidal volume even in most severe ARDS cases or in severe ventilation disorders such as bronchpleural fistula [Kopp et al., 2006].

The concept of interventional lung assist had first been mentioned in 1965 by Rashkind. He described a promising canine trial using a small disposable device as a lung substitute. Primed with saline, it was interposed between a single femoral artery and vein, with the cardiac action as the sole pumping mechanism. The device was fabricated from concentric plastic cylinders as indicated in Figure 3.4. One femoral artery and one femoral vein were cannulated, using the largest-caliber, thinnest-wall cannula that can be inserted. The arterial blood was mixed with a continuous stream of oxygen and directed into the central cylinder of the artificial lung. The mixture then flowed over and under subsequent cylinders, was de-bubbled in the outer two cylinders with the aid of a plastic sponge coated with an anti-foaming agent, and returned by gravity into the femoral vein. Rashkind obtained about 40% of the cardiac output from one femoral artery. Flows of this order of magnitude seemed to be sufficient to maintain total respiratory function [Rashkind et al., 1965].

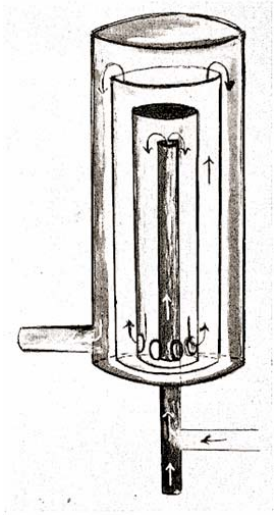

Figure 3.4 Pumpless oxygenator
(Source: Rashkind et al., 1965, courtesy Elsevier)

In 2000, Reng et al. [2000] reported clinical use of a device developed for pumpless use. The technique seemed attractive because of its simplicity and independence from machines. Since then several devices have been developed.

Novalung

The safety and feasibility of the first commercially available pumpless lung assist device, Novalung interventional lung assist (ILA) has been shown in more than 150 clinical applications (see Figure 3.5). In the vast majority of patients treated with ILA, this treatment modality has been an adjunct to mechanical ventilation that allowed optimized lung protective ventilation (to minimize ventilator-associated lung injury, and to ameliorate and eliminate the inflammatory process that is enhanced by mechanical ventilation), thus giving the lung time to heal. However, in a few cases, interventional lung assist has been employed without mechanical ventilation in the awake, nonsedated patient. The application is based on a low resistance lung assist device designed for pulsatile blood flow with tight diffusion membranes and a protein matrix coating. The gas exchange surface amounts to 1.3 m^2. It allows complete removal of arterial CO_2 and significant oxygenation of the blood. Pressure gradient across the device and cannulae is sufficiently low to omit a blood pump.

Figure 3.5 Novalung interventional lung assist device
(Source: http://www.novalung.com, *courtesy NovaLung GmbH)*

Nevertheless, the blood gas exchange capacity, especially the oxygenation capacity, of ILA is limited in comparison with pump-driven ECMO for two reasons. First, the flow through the artificial lung membrane is restricted. Because with ECMO flow rates of 4–6 $L \cdot min^{-1}$ can be achieved, physically ILA will never be equally effective. Second, the establishment of an arteriovenous shunt limits the oxygen transfer capacity, since pre-oxygenated blood passes the artificial membrane. Nevertheless, ILA exerts a most effective carbon dioxide removal and a moderate increase in oxygenation in severe ARDS [Bein et al., 2006]. When used in

non-adult patients small cannulae must be used which limit the blood flow to as little as 1 $L \cdot min^{-1}$ [Ruettimann et al., 2006]. (see Figure 3.6 and 3.7). During the use of the device heparin has to be applied continuously [Matheis, 2003]. The device is registered (CE mark) for use up to 29 days [http://www.novalung.com].

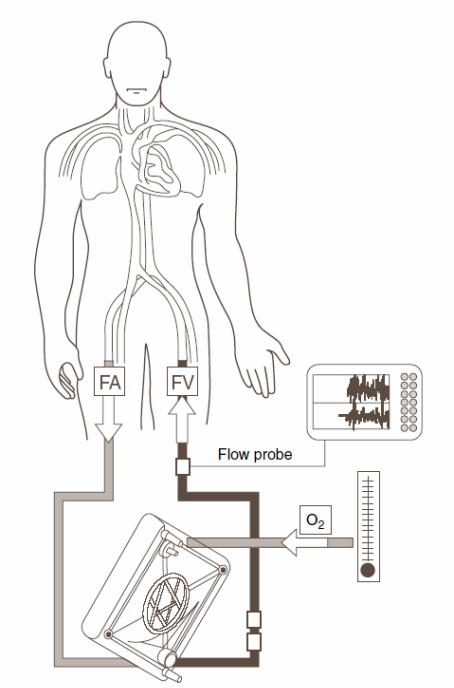

Figure 3.6 Application of the Novalung
(Source: Zimmermann et al., 2005, courtesy Oxford University Press)

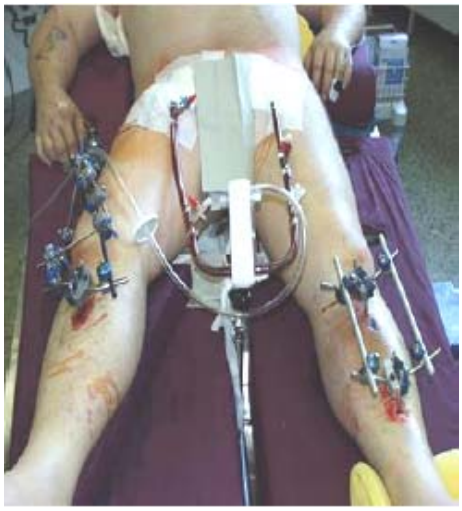

Figure 3.7 Application of the Novalung
(Source: Bein 2004, courtesy Springer Verlag)

Total artificial lung (TAL)

Several research groups are developing total artificial lung (TAL) devices for treating chronic respiratory failure, primarily as bridge-to-lung transplant devices. Whereas implantable total artificial lungs that can be placed in the thoracic or abdominal cavities may be an ultimate goal, the initial implementation and testing of TALs appears to favor paracorporeal applications, with the TAL external to but immediately attached to the patient. The attachment mode of the TAL is an important design consideration, and in-series, in-parallel, and hybrid configurations have been studied. The in-series configuration connects the artificial lung to the proximal pulmonary artery, diverting all the cardiac output through the device and returning it to the distal pulmonary artery immediately upstream of the natural lungs. Although this mode enables the natural lung to be an effective embolic filter, the mechanical load on the right heart increases because it must provide the pumping energy for blood flow through both the natural and the artificial lung. The in-parallel configuration attaches the artificial lung between the pulmonary artery and the left atrium so that only a fraction of the blood flow diverts through the artificial lung. The fraction of blood flow through the artificial lung depends on its impedance relative to that of the natural lung. The in-parallel configuration has the clear advantage that the right heart workload is reduced, but only a fraction of total cardiac output receives respiratory support from the artificial lung and that fraction is not exposed to the metabolic and filtering functions of the natural lung. The hybrid configuration attaches the inlet of the artificial lung to the proximal pulmonary artery, and uses a split return to the distal pulmonary artery (and natural lung) and to the left atrium. The hybrid configuration allows all the cardiac output to flow through the artificial lung with less resistance than the in-series configuration, and also allows greater flow through the

natural lung than the in-parallel configuration. Patients with a weak or failing right ventricle would require either the in-parallel or hybrid configurations because of the reduced power required for adequate perfusion of the artificial lung and natural lung.

BioLung

The first total artificial lung, the BioLung (MC3 Inc.) has undergone intensive bench testing and animal trials. This device could eventually help lung transplant candidates stay alive and mobile for six months or more outside the hospital, and allow them to stay healthy enough to remain at the top of the transplant list. It may also prove suitable for patients with end-stage COPD, pulmonary fibrosis or cystic fibrosis. The BioLung was shown to produce better survival and less lung injury than a conventional ventilator in five-day tests on damaged sheep lungs. The device prototype is well tolerated in series with the normal sheep pulmonary circulation. The BioLung decreased ventilator-induced lung injury to improve five-day survival. Six of eight sheep with the BioLung versus one of six with volume-controlled mechanical ventilation survived. Hemodynamic parameters with the BioLung remained stable, and the BioLung showed a very low pressure gradient. Just like ILA, the BioLung uses no mechanical pump, instead relying on the heart's own pumping force to send blood from the pulmonary artery through the device. The device is rigidly housed and has a very low resistance to blood flow. When used in series with the lungs, this device resulted in a 50% incidence of right heart failure in sheep. These results have been improved by a new design for the device, based on computer modelling and prototyping. The alterations have reduced the device's size, made it more flexible and improved blood flow, thereby enhancing the lung's performance and reducing the risk of clotting and infection [Lick and Zwischenberger, 2004]. Matching the impedance of an artificial lung for pulmonary replacement to native pulmonary impedance is important in preventing right ventricular dysfunction. Further development, therefore, addressed this problem by introducing an inflow compliance chamber, an inlet blood separator and modification of the artificial lung outlet geometry, all to reduce resistance and mimic the compliance of the pulmonary vascular bed. This type of device will initially be used in a paracorporeal fashion, i.e., with grafts connected to an extracorporeal artificial lung. This allows safe and rapid nonsurgical exchange, while an implanted artificial lung would require surgery to replace the device. Therefore, the treatment intervals will not necessarily be limited by the durability of the individual device. Artificial lungs will initially require an extracorporeal oxygen source such as a concentrator or a tank [Matheis, 2003].

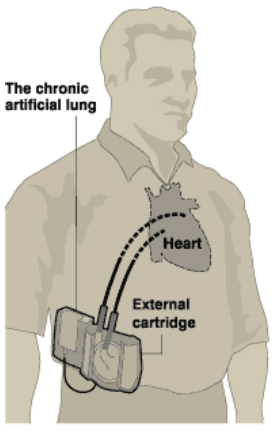

Figure 3.8 Chronic artificial lung
(Source: Spice 2001

Chronic Artificial Lung

A paracorporeal total artificial lung for chronic respiratory support (Chronic Artificial Lung, or CAL) is under development at the University of Maryland as a continuation of earlier work at the University of Pittsburgh (see Figure 3.8). The CAL is intended as a bridge-to-transplant device with the goal of 21-day support of basal metabolic needs using a device less than 0.5 m^2 in fiber membrane area. The CAL uses active mixing from a rapidly rotating disc made of microporous hollow fiber membranes that enhance gas exchange by increasing blood flow velocity past fiber surfaces and reducing diffusional boundary layers. The disc rotates within a housing and the centrifugal motion imparted to blood enables the CAL to pump blood (which may reduce the impact of the CAL on

the right heart in its intended in-series attachment mode). The motor controller directing disc rotation can generate pulsatile or nonpulsatile flow. The CAL generated 5 l/min flow against a 100 mm Hg pressure head at 1600 rpm during steady flow in bench tests using bovine blood, but adding pulsatility to the flow decreased pumping. Published data are lacking but the gas exchange efficiency of the CAL appears promising, with 550 ml/min/ m^2 and 450 ml/min/ m^2 reported for O_2 and CO_2 exchange efficiencies, respectively, in scaled-down prototypes [Federspiel and Svitek, 2004].

Portable artificial lung for conscious patients
In June 2006 the Swansea University reported that researchers are working on a new artificial lung. The device, a blood/air mass exchanger, integrates with the body's respiratory system and is designed to breathe for conscious, mobile patients whose lungs are damaged or diseased. As a portable device, it will allow patients to recover outside Intensive Care Units and offers them a better quality of life.
Although a finished product is still some years away, the results of the research to date have been encouraging. In the medium term, the device being developed at Swansea offers a bridge to transplant, meaning that people face the operation fitter and with a greatly increased chance of survival. In the longer term, the device offers an alternative to transplantation, giving hope to sufferers from emphysema and cystic fibrosis.
[http://wvn.ja.net/news_centre/LatestResearch/Headline,6318,en.asp]
The device differs from current extracorporeal life-support systems in that it uses only natural air (rather than bottled or piped oxygen). It is also integrated with the natural respiratory control system so that transfer rates of oxygen and carbon dioxide respond naturally to physical activity. In this way, patients can maintain a high level of physical fitness whilst their lungs recover, or they await lung transplant. Variants of the device range from an easily reversible fully external device to a prosthetic lung. The emphasis throughout is on a fully portable device that allows patients mobility unconnected to piped gases or complex intrusive monitoring.
[http://www.bit.or.at/irca/bbsshow8.php?ref1=06%20GB%20WADA%200F9K&vQuelle=KMT&cc=]

Portable extra corporeal membrane oxygenator
Disadvantages of the ECMO are given in the introduction. Three new designs are worth mentioning, because the problems are given due consideration. Two of them are much smaller than the 'classic' ECMO unit, which makes them portable. They incorporate a blood pump, but the pump is designed to prevent damage to the blood cells and is expected to give little problems. Heat exchangers can be omitted. The third design is a modular ECMO for use in the operating theater. It's main advantage over 'classic' ECMO is the modular design that allows the adoption of the capacity of the device to the needs of the patient.

Hexmo
Hexmo is a miniaturized extracorporeal membrane oxygenator (see Figures 3.9 and 3.10). The integration of a small rotary blood pump into the centre of the oxygenator reduces the amount of tubing and connectors in the system. Blood is convectively warmed by the pump motor housing, thus the use of a heat-exchanger can be avoided. This compact design reduces surface and priming volume and allows better handling, especially in critical situations. The blood inlet and outlet are both located at the same side of the oxygenator. This particular design allows the oxygenator housing to be directly connected with a double lumen catheter and facilitates short blood lines and closer placement to the patient. In clinical use the double lumen cannula can be introduced minimally invasively via the femoral vein into the inferior

vena cava. venous blood enters the cannula in the liver region. After passing the oxygenator, oxygenated blood flows back to the cardiovascular system close to the right atrium. HEXMO presents an oxygenation system that opens the way to mobile application, emergency use and cost reduction [Cattaneo et al., 2004].

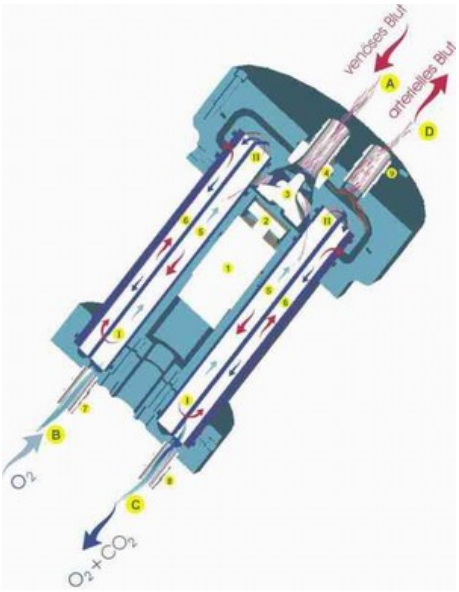

Figure 3.9 Hexmo construction
(Source: Cattaneo 2004, courtesy SAGE Publications Ltd)

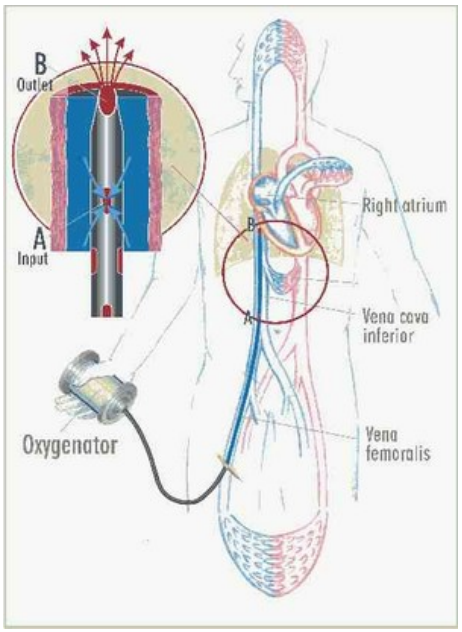

Figure 3.10 Hexmo operation
(Source: Cattaneo 2004, courtesy SAGE Publications Ltd)

Integrated heart-lung assist device (IHLAD)
The integrated heart-lung assist device (IHLAD) comprises a centrifugal pump and an artificial lung, which is located around the pump, in an all in one system. The special membrane employed precludes plasma breakthrough in extended use. The entire blood contacting surface is treated with covalent heparin bonding to impart good antithrombogenicity. Heparin bonded prototypes could be continuously perfused without systemic anticoagulation as long as 36 days in a venoarterial bypass chronic animal study using goats.
The artificial lung component consists of a polyolefin made special hollow fiber membrane, in which micropores are blind ended at the blood contacting surface to form a thin dense layer of less than 0.2 µm thickness. Direct blood-gas contact is thus completely eliminated. The entire blood contacting surface of the IHLAD is treated with covalent heparin bonding. This prominent thromboresistant surface modification will provide the IHLAD the advantage of avoiding bleeding by minimizing the necessity of systemic anticoagulation [Tatsumi et al., 1999].

Developments in materials
Membrane permeance can play an important role when coated or composite hollow fiber membranes are used to prevent plasma wetting in artificial lungs, a process in which blood plasma infiltrates the microporous walls of hollow fibers. Plasma wetting is a common problem when extracorporeal oxygenators are used in extended respiratory support and can lead to device failure within days. Plasma wetting results primarily from phospholipids, lipoproteins and/or proteins in blood that adsorb onto the fiber polymer surfaces at the plasma interface, rendering the interface hydrophilic and allowing for wetting of the pores by either partial or complete plasma infiltration. Plasma infiltration markedly diminishes the

membrane permeance, because relatively rapid gas phase diffusion is replaced by diffusion through stagnant plasma within fiber pores. The membrane permeance for a completely wetted hollow fiber is a 100,000-fold lower than for gas-filled pores. Thus even partial plasma infiltration into fiber membranes can significantly reduce membrane permeance and degrade artificial lung performance. Composite hollow fibers incorporate a thin nonporous polymer layer as a true membrane or 'skin' on the microporous fiber surface. The true membrane blocks infiltration of plasma into pores and is a key functional requirement of artificial lungs for longer-term respiratory support. Composite hollow fiber membranes are made either by coating an existing microporous fiber with a thin nonporous polymer (a true composite hollow fiber) or by modifying the fabrication of the microporous fiber itself to seal off pores at the surface (an asymmetric hollow fiber). The nonporous polymer skin that prevents plasma wetting also diminishes membrane permeance because a nonporous polymer can present an impediment to gas diffusion.

Material induced activation of blood could theoretically be reduced through the addition of coatings to the surfaces of the artificial lung, that release nitric oxide. Physiologically, nitric oxide acts as a local inhibitor of platelet adhesion and activation. Such a surface may be required for artificial lungs, as it may prevent lack of platelets, platelet dysfunction, and clot formation that has complicated the use of extracorporeal circuits for many years [Zwischenberger, 2001]. The university of Michigan is working on polymers that contain tiny silica particles that release low levels of nitric oxide gas.

Alternative oxygen generation

A patent describes a technology to generate oxygen from the water in blood plasma by photolytic decomposition of the water molecule into O_2 and H_3O^+. It is description of principle, without a clear indication of a working system. The system requires a blood pump, a UV-laser to illuminate the catalyst that is in contact with the bloodstream, a gas sorption device to remove the formed H_2 and another gas sorption device to remove CO_2 from the bloodstream. [http://www.freshpatents.com/Oxygen-generation-in-whole-blood-by-photolytic-activation-dt20051201ptan20050265894.php]
The technology as described in the patent is complicated and a long way from practical application.

As an alternative gas exchange concept, the feasibility of a bioregenerative artificial lung has been shown. The photosynthetic capacity of algae (*Chlorella pyrenoidosa*) was maximized at a cell density of 25 million cells/ml to serve as an oxygen producer and CO_2 remover. A reservoir containing blood was interfaced with this system via a gas transfer membrane [Basu-Dutt, 1997].
The principal of this technology has been demonstrated; it is possible to oxygenate blood from a tank with algae. The volume of the tank was about 200 liters and the researchers estimated that the volume can be reduced to 35 liters, which is still bulky. The algae were illuminated with special lamps, requiring 700 watts of energy. In the current stage of development this technology has no advantages over oxygen supply from a tank or concentrator.

3.1.2 Applications in the Netherlands

UMMOX

The Utrecht Micro Engineering Competence Centre, a division of the Utrecht Technical High School developed a concept for a modular oxygenation system for open-heart surgery: the UMMOX system (see Figure 3.11). The system can be adapted to oxygen needs of the patient, based on the body weight. The oxygenator can be made as small as necessary, thus minimizing the priming volume and the contact surface for blood. Three basic units have been developed, suitable for 4, 10 and 30 kg of body weight. Using these modules a custom-made oxygenator can be assembled to suit the needs of virtually all patients; from babies to obese adults.

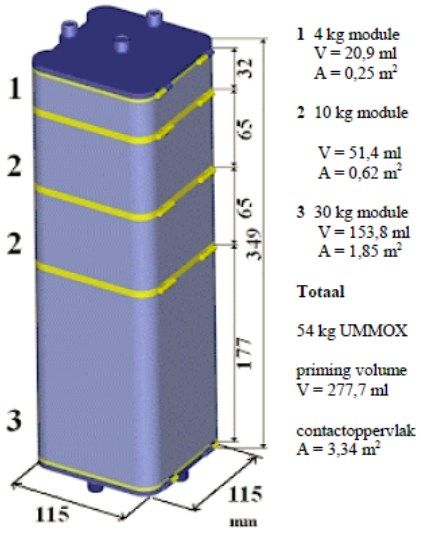

Figure 3.11 UMMOX
(Source: http://www.umecc.nl/, *courtesy Hogeschool Utrecht)*

The oxygenator consists of sheets with micro-channels. Two sheets are stacked on top of each other with the micro-channels facing each other in a perpendicular orientation to allow for cross-flow, separated by a gas-permeable membrane. One of the sheets facilitates the blood flow, while the other sheet is flushed with oxygen.

The design concept was presented in 2004 at the 5th symposium 'MicroSystems in Practice'. In a personal communication in January 2007, mr. Sillen of the Utrecht Technical High School stated that the device is still under development. The efficacy of gas-exchange over the membrane is expected to double. [http://www.umecc.nl/ummox.html]

3.2 Cell/tissue-based approach for function recovery

Cell/tissue based approaches either work towards combining a medical device with cells or to use a tissue engineering or cell therapy approach to produce new lung tissue.

One of the ideas to improve the blood compatibility of the gas exchange fibers that are used in many of the described artificial lungs, is the application of endothelial cells. Endothelial cells line the blood vessels in the body which the body recognizes as its own. One way to counter the problem of biocompatibility would be to use them in these devices. A physician would harvest endothelial cells from the patient and then seed the micro fabrication module and have the cells line the blood passages. This would lead to a completely biocompatible surface in the patient's blood stream. This idea of coating the device with a patient's own endothelial cells will happen most likely in a 10-20 year time frame. The development of better basic hollow fiber membranes is expected to happen in a five to ten year time frame [Madhani, 2004].

The structural complexity and cellular diversity of the lung, coupled with the slow turnover rates of pulmonary epithelium make the lung a difficult target for cell therapy. Besides the trachea, major bronchi containing ciliated and mucous secretory cells and bronchioles containing Clara cells, which detoxify inhaled pollutants, the most important cells for the process of gas exchange are the cells that form the alveoli. The alveoli are lined by two epithelial phenotypes; flattened squamous (type I) and cuboidal (type II) pneumocytes.

Whereas the type I pneumocytes are responsible for the gas exchange, the type II pneumocytes are critical for maintaining alveolar homeostasis, clearing the alveolar airspace of oedema and secreting pulmonary surfactant to lower surface tension and prevent airway collapse. Turning stem cells into cells needed for pulmonary gas exchange may allow the regeneration of damaged lung tissue, and ultimately the creation of artificially grown lungs may make it possible to repair lungs that have been damaged by disease, by implanting fully functioning cells to repopulate damaged areas.

3.2.1 State of development

In the literature, there are several approaches discussed regarding potential lung cell therapy. These approaches can roughly be divided into the following groups:

I) Targeted activation or administration of endogenous stem cells,
II) Creation of pulmonary tissue constructs in vitro,
III) Biohybrid lung that combines a medical device with living cells [Bishop and Rippon, 2006].

The preference for a specific approach is likely to depend on the type of lung disease, including the amount of damage and the type of lung tissue that is damaged. Furthermore, the extracellular matrix affected in a specific disease plays a major role. E.g. in pulmonary fibrosis there is excess accumulation of collagen and other matrix proteins. Remodeling of this matrix would be required to restore lung function to the affected airspaces. Emphysema, on the other hand, is characterized by loss of alveolar units as a result of matrix proteolysis followed by cell death. Restoration of functional alveolar units may require the addition of a scaffold material to allow migration of stem cells and formation of alveoli [Stripp and Shapiro, 2006].

I) Targeted activation or administration of endogenous stem cells

The activation or administration (e.g. by infusion) of stem cell pools is suggested to augment the existing repair mechanisms of the body. Stem cells that are present in the lung itself are a potential source. Several research groups have shown the presence of cells obtained from lung tissue that possess a high proliferative capacity. It has been demonstrated that in the proximal airways ciliated cells have a proliferative capacity that can affect repair, whereas Clara cells perform this function in the distal airways [Hong et al., 2004]. In the alveoli, type II pneumocytes represent the proliferative population and are able to transdifferentiate into type I pneumocytes following injury of the alveolar wall [Bowden, 1984]. It has been shown that these type II pneumocytes can be sorted on the basis of E-cadherin expression [Reddy et al., 2004]. Another source of stem cells that can differentiate in vitro to bronchiolar and alveolar epithelium has been reported to be isolated from the lungs of adult sheep and rats. These cells are extremely small with a very low oxygen demand and appeared exceptionally resistant to damage, leading the researchers to call them ‘spore-like’ [Vacanti et al., 2001]. Also circulating stem cells are reported to be a potential source for lung cell therapy [Yamada et al., 2005]. Importantly, it has been reported that circulating bone marrow-derived stem cells can home to a damaged respiratory epithelium undergoing regeneration [Kotton et al., 2001]. It has been demonstrated that Y chromosome-containing male cells were present in the lungs (lining of the lung) of male patients who had received a lung transplant from a female donor. These observations suggest that, as occurs in rodents, the epithelium of the adult human lung has the capacity to renew itself, using cells recruited from extrapulmonary sources, including the bone marrow [Albera et al., 2005]. Burnham and colleagues have reported the relationship between circulating endothelial progenitor cells (EPC) and survival in patients with acute lung injury. They found that the number of circulating EPCs increased in patients with acute lung injury, indicating that EPCs are mobilized from the bone marrow

under inflammation conditions. Since these EPCs seem to play a critical role in survival from acute lung injury, it has been suggested that these cells can be used as a therapeutic tool [Kobu, 2005]. Also bone marrow-derived mesenchymal stem cells have been reported to enhance the repair mechanism of damaged lung tissue. The mesenchymal stem cells were associated with the differentiation into specific and distinct lung cell phenotypes [Rojas et al., 2005]. Nevertheless, despite the research in stem cells and progenitor cells, only few studies have been reported that demonstrate an augmented lung repair due to an up-regulated natural repair process initiated by a cell therapy approach. An efficient way of recruiting, mobilizing and differentiating relevant stem cell and progenitor pools should be developed before this approach can be introduced as a clinical treatment to repair damaged lung tissue.

II) Creation of pulmonary tissue constructs in vitro

The use of in vitro cultured constructs theoretically allows the repair of more extensive tissue damage. The majority of this lung tissue engineering has been performed by using embryonic stem cells in view of their pluripotency and proliferative capacity. The embryonic stem cells have been cultured on several substrates, such as matrices based on poly(L-lactic acid) [Moscato et al., 2006], Matrigel hydrogel [Mondrinos et al., 2006] and collagen-glycosaminoglycan [Chen et al., 2005]. The formation of Clara cells, type I pneumocytes and type II pneumocytes has been demonstrated by culturing murine embryonic stem cells [Rippon et al., 2006]. Nevertheless, it has proven difficult to obtain lung epithelial cells yields of more than a few percent in the cultures of differentiated embryonic stem cells. In order to increase this percentage, several approaches such as cell culture at an air-liquid interface [Coraux et al., 2005], the use of a culture method originally used to convert fibroblasts into T cells using T cell extracts [Hakelien et al., 2002], and the use of the inductive power of fetal lung mesenchyme [Van Vranken et al., 2005] has been explored. To proceed to clinical applications, the technology developed by using the murine embryonic stem cells must be transferred to human embryonic stem cells. Anne Bishop and her team from London (Imperial College London Tissue Engineering and Regenerative Medicine Centre at Chelsea and Westminster Hospital) now plan to begin development of a living construct, using bioactive foams and scaffolds. They will provide a frame on which the cells can grow and then be transplanted. Bishop and her colleagues took mouse embryonic stem cells, placed them in a specialized growth system and encouraged them to change into cells that line the lung where O_2 is absorbed and CO_2 is excreted. They have begun to replicate their findings using human embryonic stem cells. The transformed cells could help reline the lungs in patients who had lung damage or in premature infants whose lungs were not fully matured. Unlike transplanted cells from a donor, these cells could be developed so the body would not reject them. Currently, these cells are grown on natural and artificial scaffolds, and the aim is to construct lung tissue that could be used in implantation.

Recently, type II pneumocytes have been successfully differentiated from human embryonic stem cells by Samadikuchaksaraei et al. [2006]. However, due to ethical aspects it is not expected that this approach will be introduced in the clinic for a long time. Another possibility is the use of adult stem cells, as mentioned in the paragraph above. Especially healthy non-pulmonary somatic stem cells would be a potential source due to the easy accessibility and their abundance. The research group at the University of Minnesota (Minneapolis, Minn., USA) has reported for the first time the differentiation of human umbilical cord blood (UCB) stem cells (termed Multi-Lineage Progenitor Cells (MLPC; BioE, Inc., St. Paul, Minn., USA)) into respiratory epithelial cells, specifically type II alveolar cells. They were able to successfully induce essentially all MLPCs into type II alveolar cells [Berger et al., 2006]. Also results by using somatic lung progenitor cells (SLPC; high percentage of CD34 and CD117 positive cells) from mammalian lung tissue grown onto synthetic polymer scaffolds have

been published. The cells were shown to express lung-specific markers for Clara cells, pneumocytes and respiratory epithelium and organized into identifiable pulmonary structures (including those similar to alveoli and terminal bronchi), with evidence of smooth muscle development (see Figure 3.12).

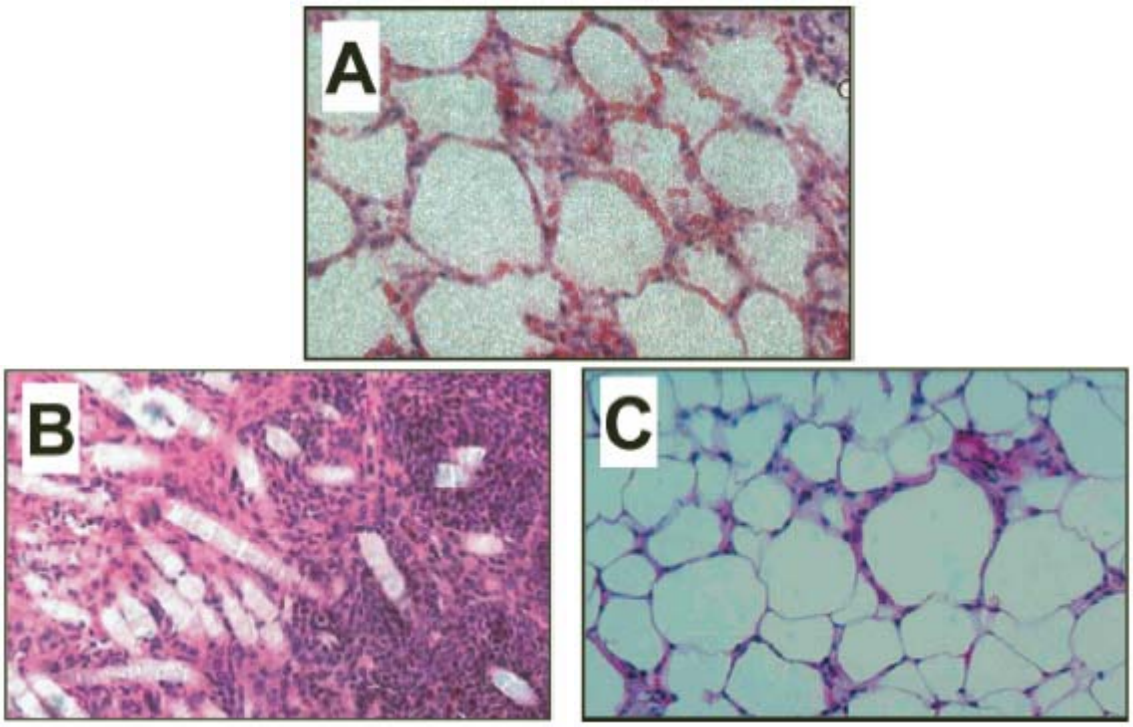

Figure 3.12. In vitro tissue-engineered lung. Sections of engineered lung produced after implantation of ovine somatic lung progenitor cell (SLPC)/polymer tissue constructs on the backs of nude mice. Photomicrographs of hematoxylin and eosin stained sections of normal lung (A) and tissue-engineered lung (B and C). (B) Engineered tissue grown using ovine SLPC/polyglycolic acid constructs. (C) Engineered tissue grown using ovine SLPC/30% PF-127 constructs (magnification X200) *[Cortiella et al., 2006]*.

Also adipose tissue-derived stromal cells cultured on polyglycolic acid felt sheets are potential constructs that can be used in pulmonary cell therapy. These cells produce a large amount of angiogenic factors, including hepatocyte growth factor, which is known to be a potent regenerative factor generated after a lung injury. It has been demonstrated that the alveolar and vascular regeneration of lungs (which have undergone lung volume reduction surgery) covered with a adipose tissue-derived stromal cell-coated sheet is significantly faster as compared to the regeneration in rats that underwent lung volume reduction surgery alone [Skigemura et al., 2006]. Nevertheless, the results are yet far from optimal and the time frame for getting such an approach into the clinic remains unknown (at least 5-10 years). The first barrier to overcome is to optimize the culture techniques in order to improve the control of the proliferation and the differentiation concerning the relevant cell types. And finally, it is the big question if these cultured cells will lead to functional airway and alveoli in vivo. As stated before, the lung is composed of a complicated three-dimensional structure requiring precise alignment of airspace and vasculature to exchange gas. In addition, to ensure long-term maintenance of the engineered tissue, the tissue-coated structures should harbor stem cell niches with their associated stem cells. Studies should be conducted to define cellular and molecular mechanisms of stem cell maintenance in vivo and the unique properties of stem cell niches that supply this supportive microenvironment [Stripp and Reynolds, 2005].
A more simple approach, regarding the type of lung tissue, which has been clinically applied in one patient at the Heidehaus hospital in Hannover, Germany, concerns a cell-coated

tracheal substitute (see Figure 3.13). The process to engineer the tracheal substitute comprised the isolation of muscle cells and fibroblasts from a biopsy. The cells were subsequently seeded on a collagen matrix generated from a decellularized porcine jejunal segment. After an incubation period, the cells started to remodel the xenogenic matrix and replaced it with autologous connective tissue. Within 4 weeks an autologous bioartificial implant was formed and had been implanted in a patient with an extensive tracheo-bronchial insufficiency. The tissue was incorporated into the native trachea without signs of infection, rejection or necrosis. After implantation, the luminal patch surface was reseeded with functional respiratory epithelium. There was no evidence of granulation tissue formation at the implantation site [Macchiarini et al., 2004]. In the coming years long-term experimental implantations in animals and humans can be expected.

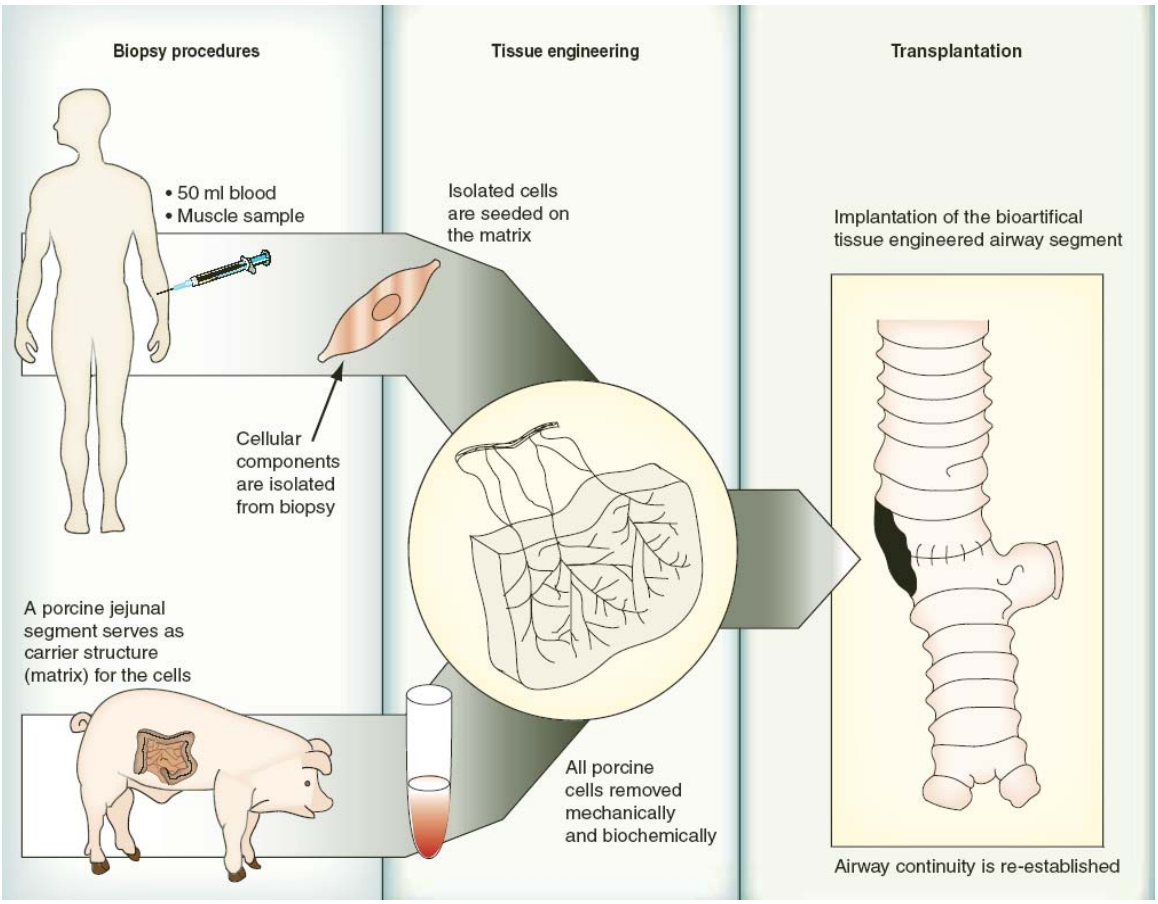

Figure 3.13. Process of bioartificial tracheal patch generation applied in the clinical setting *[Walles, 2004]. Reproduced with permission of Future Drugs Ltd.*

III) Biohybrid lung that combines a medical device with living cells

The culturing of cells in a medical device is another approach to create an artificial lung. Researchers at the Tissue Engineering and Regenerative Medicine Centre (TERM) of Imperial College London are collaborating with Novalung and NovaThera in order to increase the efficiency and lifespan of already existing lung-assist devices [www.novalung.com]. They have developed a novel process for the controlled and directed differentiation of both murine and human embryonic stem cells into Type II Pneumocytes. Furthermore, they have demonstrated that the Type II pneumocytes can be grown on synthetic polymer scaffolds, including the surface of capillary fibers. This had opened up the possibility of using such cells to augment the functionality of synthetic membrane based lung bypass devices. This approach has been applied by using the iLA Membrane Ventilator. The

iLA Membrane Ventilator extracts blood from the body via the femoral artery, which is subsequently led into the device where gas exchange takes place and finally the blood is piped back into the femoral vein. Inside the device lies a series of polymer membranes which comprise a complex intermesh of synthetic capillaries, through which oxygen can be pumped. The incorporation of cultured lung cells on the surface of the polymers should improve the efficiency for gas exchange. As stated by NovaThera, clinical trials are hoped to begin within one and a half years. As the technology develops, the company intends to develop a tissue coated device that can be implanted into the diseased lungs. However, the company has estimated that these clinical trials are 5-10 years away.
Another biohybrid system is in development by associate professor William J. Federspiel and graduate student Kristie Henchir, who are both working at the McGowan Institute for Regenerative Medicine at the University of Pittsburgh (see Figure 3.14). This biohybrid lung is based on a MEMS (micro-electro-mechanical-system) device, which is about the size of a thick credit card, that duplicates the function of the alveoli by bringing air and blood into close contact (see figure below). The MEMS is laced with micro-channels containing either air or blood, which are separated by thin membranes that mimic the alveolar wall. To prevent coagulation, the blood-containing micro-channels are lined with endothelial cells. Federspiel and his collaborators use endothelial cells from discarded umbilical cords, but have planned to use cells taken from a patient's own fat tissue in the future. Once these little units are perfected, they could be integrated into larger-scale modules that could be either implanted or worn outside the body. The group has performed research to optimize the growth and coverage of the cells on the device and persistence of the cells under dynamic conditions. Future work will focus on additional cell culture studies and optimising gas exchange characteristics of the modules. No statements were found regarding initiation of clinical trials. Nevertheless it is not expected that clinical trials will be initiated in the coming five years.

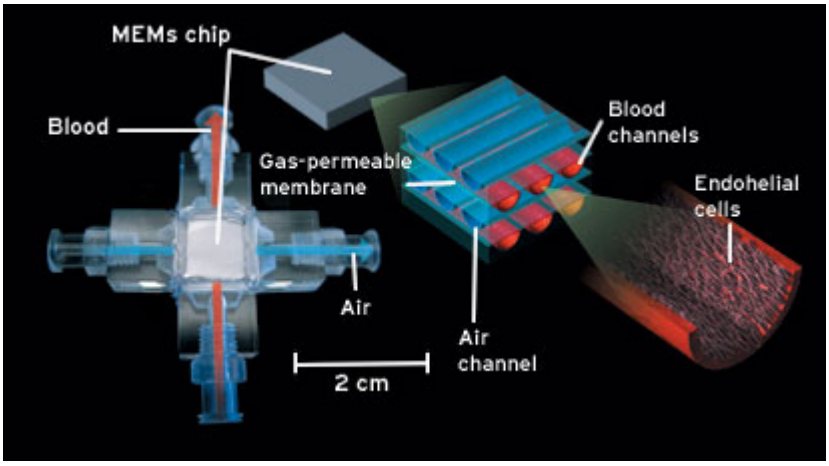

Figure 3.14 A prototype bioartificial lung under development at the McGowan Institute for Regenerative Medicine *[Reproduced with permission of Bryan Christie Design]*.

3.2.2 Applications in the Netherlands

Dutch organisations that support lung based research of academic research groups are ‘Nederlands Astmafonds’ [www.astmafonds.nl], ‘Nederlandse Longstichting (NLS)’ and ‘Nederlandse Cystic Fibrosis Stichting’ [www.ncfs.nl]. Although some of this research is focussed on the basic repair mechanism of lung tissue, no applied research is currently performed in order to develop a lung cell therapy. By our knowledge, no academic groups are planning to start the development and testing of lung cell therapy approaches in the near future.

3.3 Conclusion

The natural lung represents a remarkable organ for gas exchange, and developing an artificial lung that approaches the gas exchange powers of the natural lung is a significant engineering challenge. Current hollow fiber blood oxygenators, as used in cardiopulmonary bypass, have membrane areas ranging from 1 to 4 m^2 that are packaged much less compactly than in the natural lung, with a surface area to blood volume ratio 10 times less than in the natural lung. The effective distance that gas diffuses between blood and gas flow pathways in artificial lungs is approximately 10–30 μm, an order of magnitude greater than in the natural lung. Thus, even with using 100% oxygen gas, artificial lungs currently used or under development aim at gas exchange levels that can support resting metabolic needs in patients. None of the artificial lungs described in this report make an attempt to mimic any of the other functions or properties of the lung. The non-cell artificial lungs described in this report derive directly in a conceptual sense from the hollow fiber membrane and membrane module technology used in traditional clinical blood oxygenators. Many of these artificial lung devices will hopefully achieve some clinical success in the next five to ten years.
Regarding the cell-based approaches, several approaches are being developed worldwide. These approaches can be divided into the following categories: I) Targeted activation or administration of endogenous stem cells, II) Creation of pulmonary tissue constructs in vitro, III) Biohybrid lung that combines a medical device with living cells. All of these approaches are still in the research phase, although it has been reported that a cell coated tracheal substitute has been applied in one patient (Germany). In the Netherlands the development of approaches with living cells to reconstruct, repair or replace pulmonary tissue and function has still to be initiated. Therefore, it can be concluded that it is not expected that cell-based treatment of the lung will be applied in the Dutch clinics in the coming 5-10 years.
The challenge of biocompatibility inherent in making microvascular-scale blood channels, with an extensive blood contact area, non-thrombogenic and non-inflammatory may require the use of endothelial cells, perhaps genetically engineered for enhanced performance or for the robustness required in the application. Significant advances in tissue engineering, biomaterials, microfabrication, and bioengineering will all need to be harnessed for the technological development of future artificial lungs. Artificial lungs that will allow patients any significant level of increased metabolic activity are not on the immediate horizon. At the same time, the need for artificial lungs in the distant future may be eclipsed by significant advances in regenerative medicine that enable tissue repair and regeneration of failing lungs.

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4. Liver

The liver plays a major role in metabolism and has a number of functions in the body including glycogen storage, plasma protein synthesis, and drug detoxification. This organ is the largest gland in the human body. It produces bile, which is important in digestion. It performs and regulates a wide variety of high-volume biochemical reactions requiring specialized tissues. Medical terms related to the liver often start with hepato- or hepatic from the Greek word for liver, *hepar*.
The liver is among the few internal human organs capable of natural regeneration of damaged or lost tissue; as little as 25% of remaining liver can regenerate into a whole liver. This is predominantly due to the hepatocytes acting as unipotential stem cells (i.e. a single hepatocyte can divide into two hepatocyte daughter cells). There is also some evidence of bipotential stem cells, called oval cells, which can differentiate into either hepatocytes (liver cells) or cholangiocytes (cells that line the bile ducts).
The liver has a large number of functions:

- The liver produces and excretes bile required for emulsifying fats. Some of the bile drains directly into the duodenum, and some is stored in the gallbladder.
- The liver performs several roles in carbohydrate metabolism:
  - Gluconeogenesis; the synthesis of glucose from certain amino acids, lactate or glycerol
  - Glycogenolysis; the breakdown of glycogen into glucose
  - Glycogenesis; the formation of glycogen from glucose
  - The breakdown of insulin and other hormones
- The liver is responsible for the mainstay of protein metabolism.
- The liver also performs several roles in lipid metabolism:
  - Cholesterol synthesis
  - The production of triglycerides (fats).
- The liver produces coagulation factors I (fibrinogen), II (prothrombin), V, VII, IX, X and XI, as well as protein C, protein S and antithrombin.
- The liver breaks down haemoglobin (that results from the breakup of dead red blood cells), creating metabolites (bilirubin and biliverdin) that are excreted through bile.
- The liver breaks down toxic substances and most medicinal products in a process called drug metabolism. This sometimes results in toxication, when the metabolite is more toxic than its precursor.
- The liver converts ammonia to urea.
- The liver stores a multitude of substances, including glucose in the form of glycogen, vitamin B12, iron, and copper.
- In the first trimester fetus, the liver is the main site of red blood cell production. By the 32nd week of gestation, the bone marrow has almost completely taken over that task.
- The liver is responsible for immunological effects- the reticuloendothelial system of the liver contains many immunologically active cells, acting as a ‘sieve’ for antigens carried to it via the portal system.

Diseases of the liver
Many diseases of the liver are accompanied by jaundice caused by increased levels of bilirubin in the circulatory system. The most important diseases of the liver are the following:

- Hepatitis, inflammation of the liver, caused mainly by various viruses but also by some poisons, autoimmunity or hereditary conditions.

- Cirrhosis is the formation of fibrous tissue in the liver, replacing dead liver cells. The death of the liver cells can for example be caused by viral hepatitis, alcoholism or contact with other liver-toxic chemicals.
- Hemochromatosis, a hereditary disease causing the accumulation of iron in the body, eventually leading to liver damage.
- Cancer of the liver (primary hepatocellular carcinoma or cholangiocarcinoma and metastatic cancers, usually from other parts of the gastrointestinal tract).
- Wilson's disease, a hereditary disease which causes the body to retain copper.
- Primary sclerosing cholangitis, an inflammatory disease of the bile duct, autoimmune in nature.
- Primary biliary cirrhosis, autoimmune disease of small bile ducts
- Budd-Chiari syndrome, obstruction of the hepatic vein.
- Gilbert's syndrome, a genetic disorder of bilirubin metabolism, found in about 5% of the population.
- Glycogen storage disease type II. The build-up of glycogen causes progressive muscle weakness (myopathy) throughout the body and affects various body tissues, particularly in the heart, skeletal muscles, liver and nervous system.

Clinically, liver failure carries a high mortality, about 90% [Park and Lee, 2005]. The impairment or loss of liver functions such as detoxification, metabolism, and regulation leads to life-threatening complications, including kidney failure, encephalopathy (‘hepatic coma’), cerebral edema, severe hypotension, and susceptibility to infections culminating in multiple organ failure in patients with acute liver failure (ALF). The only established effective treatment for such patients is liver transplantation, either from living or non-living donors. However, currently, one-third of these patients die while waiting for transplants because of the organ shortage.

A considerable amount of work has been done over many years to develop effective liver support devices in order to bridge the failing liver to transplantation or as a bridge to recovery, as liver failure is potentially reversible because of liver regeneration. The development of these devices has followed two different strategies. The first approach is based on detoxification functions using membranes and adsorbents. The second approach comprises biological devices using viable cells [Onodera, 2006].

4.1 Medical device-based approach for function recovery

For many years it was assumed that toxins which cause hepatic coma are small molecules that could be removed by hemodialysis or hemofiltration. Currently, a large number of substances that accumulate in the blood in case of hepatic failure have been identified as causing neurological problems, aggravation of injury to other organs (e.g. kidney), impairment of the activity of residual ‘healthy’ liver cells and the regeneration of the diseased cells. These toxic molecules include not only small molecules (like ammonia, bile acids), but also mediators of inflammation (e.g. cytokines) and vasoactive substances and cell growth inhibitors. The purpose of the blood detoxification/purification treatment in liver failure is the removal of this wide range of different toxins [Rozga, 2006] and to this end various combinations of solid (charcoal, resin) and fluid (albumin) exchange media and membranes (material, pore size) are being used.

4.1.1 State of development

Hemodialysis

At present, hemodialysis is the standard therapy for end stage renal disease. The artificial kidney has had a profound influence on the development of the artificial liver. In 1958, Kiley et al. [1958] reported the first use of hemodialysis to treat liver failure caused by liver cirrhosis. Five patients suffering from ammonia intoxication were treated with hemodialysis. Although four patients had symptomatic improvement of their neurological status, none achieved long-term survival. Opolon et al. [1976] reported the use of polyacrylonitrile membrane hemodialysis to treat acute fulminant hepatitis in an uncontrolled clinical study. This membrane removed many of the higher molecular weight molecules associated with encephalopathy, up to a molecular weight of 15000. The recovery from encephalopathy was statistically significant; however, the changes in survival rate were not. Removal of these substances was not able to affect the survival of patients with liver failure, although hemodialysis did work well on renal failure associated with liver failure [Onodera et al., 2006].

Charcoal hemoperfusion

Direct hemoperfusion is a method of removing toxins from the blood by using adsorbents. Charcoal hemoperfusion can remove many water-soluble molecules associated with encephalopathy. It was first reported as a successful treatment for severe barbiturate overdose. Since then, charcoal hemoperfusion has been used widely to treat hepatic failure. However, controlled clinical trials using coated charcoal did not show improvement of the long-term survival of patients with fulminant hepatic failure [Onodera et al., 2006].

Plasma exchange

Plasma exchange is a method of separating the plasma element from the material element of blood by using a hollow fiber filter made of cellulose-diacetate and polyethylene membrane. Lepore and Martel first performed plasma exchange therapy for liver failure in 1970. The rationale of plasma exchange for treatment of hepatic failure is to remove toxins and to supply lacking or defective components such as albumin and clotting factors. Plasma exchange was associated with the reversal of hepatic coma and a statistically significant improvement of coagulation indices for patients with acute hepatic failure. However, both the risk of infection and the need for large supplies of costly fresh frozen plasma has made it difficult to continue the plasma exchange therapy for liver failure in recent years. Controlled trials are needed to establish the beneficial effects of plasma exchange on patient survival [Onodera et al., 2006].

Hemodiafiltration

Based on the assumption that mid-sized molecules are responsible for hepatic coma in patients with fulminant hepatic failure (FHF), a hemodiafiltration (HDF) method using a high-performance membrane such as a large-poresized polymethylmethacrylate (PMMA) membrane was developed in 1986. In a retrospective study, patients showed complete recovery from deep coma and long-term survival in cases of severe FHF [Onodera et al., 2006].

Plasma exchange and continuous hemodiafiltration

To efficiently remove middle-molecular-weight substances such as hepatic toxins and to minimize the adverse effects associated with plasma exchange, Nitta et al. [2002] have developed a combination of slow plasma exchange in combination with high-flow continuous hemodiafiltration (See Figure 4.1).

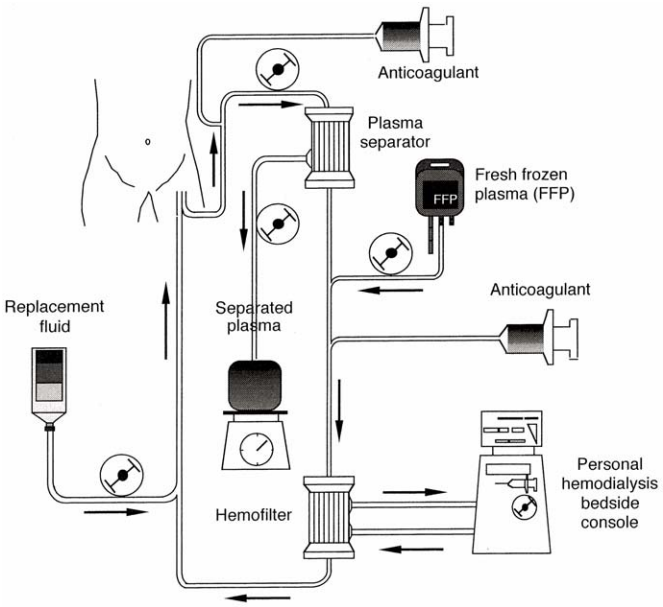

Figure 4.1. Flow diagram and operational conditions of slow plasma exchange (PE) plus high-flow continuous hemodiafiltration (CHDF).
(Source Onodera et al., 2006, courtesy of Springer, Heidelberg)

They reported a retrospective clinical study of five patients with liver failure and concluded that the adverse effects associated with plasma exchange alone for artificial liver support for liver failure could be alleviated with the combined use of plasma exchange plus continuous hemodiafiltration instead. They showed that adverse effects including hypernatremia, metabolic alkalosis, and a sharp decrease in colloid osmotic pressure can be alleviated by this procedure. This procedure is also efficacious as artificial liver support in preventing the adverse effects caused by plasma exchange (PE) and for continuous removal of hepatic coma-inducing substances. As a result, 35%–40% of FHF patients recovered with this treatment, and the remaining patients are considered for living related liver transplantation [Onodera et al., 2006].

Cryofiltration

Cryofiltration is an extracorporeal immune modulation technique originally introduced by Malchesky et al. [1980]. Cryofiltration for plasma treatment uses two filters, a plasma separator and a cryofilter (see Figure 4.2). The patient's blood is led to the first filter in which the blood is separated, and the plasma is cooled in the presence of heparin in a heat exchanger. The plasma is then filtered through a second filter made of cellulose-diacetate. The main indications are immune complex diseases including rheumatoid arthritis, systemic lupus erythematosus, polymyositis, primary biliary cirrhosis, and chronic rejection of a graft kidney. The advantage of this modality is that there is no need for massive volumes of plasma, which may cause infection. Cryofiltration combined with plasma-exchange therapy has improved persisting cholestasis, hyperbilirubinemia, and hepatic coma. However, the survival rate did not improve. The mechanism of the effects of cryofiltration may be attributed to the improvement of hepatic microcirculation caused by a reduction of plasma viscosity and activation of the bile pathway by the reduction of cryogel, including immunoglobulin and immune complexes.

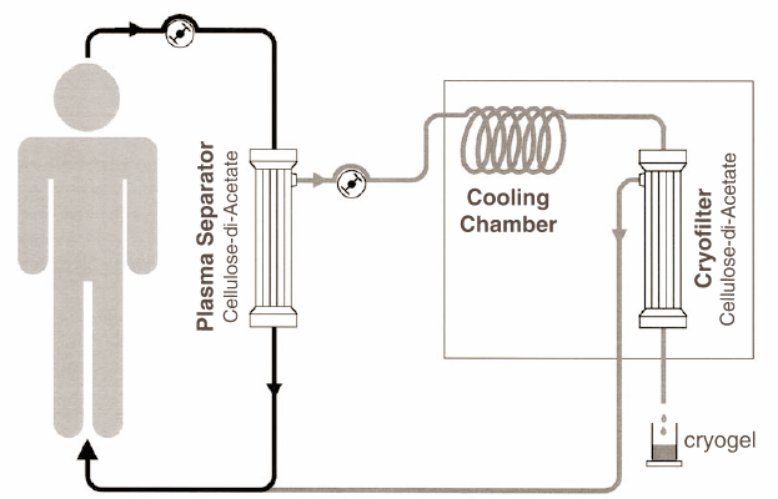

Figure 4.2. Cryofiltration system incorporating plasma separation, plasma cooling, and membrane filtration to remove cryogel. *(Source Onodera et al., 2006, courtesy of Springer, Heidelberg)*

Molecular adsorbent recycling system (MARS)
Many therapeutic procedures based on removal of toxins failed to improve patient survival. The reason for therapy failure is that most of these techniques predominantly focus on removal of water-soluble substances, whereas the accumulation of protein bound substances is unaffected. In addition, unintended removal of various growth factors delayes the process of liver regeneration. MARS system combines hemodialysis against albumin dialysate with a conventional dialysis procedure.
MARS has been shown to remove watersoluble and albumin bound low- and middlemolecular-weight toxins with high selectivity as a result of the use of a high-flux membrane, such as a polysulfone membrane. Moreover, it has an additional dialysis component for removal of water-soluble toxins. MARS uses a hollow-fiber dialysis module in which the patient's blood is dialyzed across an albumin-impregnated polysulfone membrane with a cut-off of 50 kDa while maintaining a constant flow of 600ml of 20% albumin as dialysate in the extracapillary compartment (see Figure 4.3). MARS achieves high clearances for water-soluble substances (e.g. ammonia, cytokines, creatinine, urea) because of high dialysate flow rates as well as high clearance for albumin bound substances (such as bilirubin, bile acids, and nitric oxide as an endogenous vasodilator). The advantage of MARS is that it is easy to use and inexpensive compared to bioartificial devices or standard medical therapy.

Most of the clinical studies carried out with MARS have been on patients with acute-on-chronic liver failure (ACLF), not on those with acute liver failure (ALF). In the first randomized trial of MARS, 13 ACLF patients with rapid-onset type I hepatorenal syndrome were treated with MARS or standard medical therapy, including hemodiafiltration. MARS treatment showed significantly improved survival in a controlled trial of the patients with hepatorenal syndrome. Mortality rates in the control group were 100% at day 7 compared with 63% for the MARS-treated group. The method also proved safe without serious adverse side effects. Thus, it was concluded that MARS appeared to be useful as a bridge to orthotopic liver transplantation (OLT).
The most recent randomized controlled study was performed in 24 patients with ACLF with progressive hyperbilirubinemia. MARS was associated with improved 30-day survival (11/12 vs. 6/11 in controls). Renal dysfunction and hepatic encephalopathy also improved in the MARS group. It was concluded that MARS appeared to be effective and safe for the

short-term treatment of patients with liver cirrhosis and superimposed acute injury associated with progressive hyperbilirubinemia.

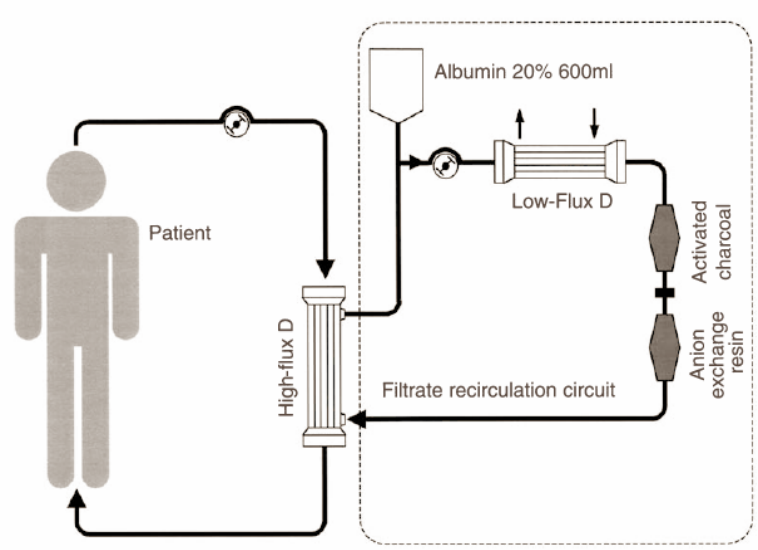

Figure 4.3. The molecular adsorbent recycling system (MARS) apparatus consists of a hollow fiber dialysis module in which the patient's blood is dialysed across an albuminimpregnated high-flux polysulphone membrane (MARSFlux), while at the same time maintaining a constant flow of albumin-rich (usually 20%) dialysate in the extracapillary compartment. The adsorbed toxins from the binding sites on the membrane pass to the albumin-binding sites in the dialysate. The dialysate is then passed through a column through which conventional dialysis/ filtration is performed across a low-flux membrane, and then perfused successively over an activated charcoal column and an anion exchange resin column to remove the albumin-bound toxins, and thus regenerate the dialysate. *(Source Onodera et al., 2006, courtesy of Springer, Heidelberg)*

In general, treatment is well tolerated and the only consistent adverse finding with the use of MARS is reduced platelet count. The major drawbacks of this system could be a higher risk of infection because of the need for a central vein catheter and the need for a very large amount of albumin.
Numerous studies have been published, with the majority of them describing the capability of MARS in removing albumin bound toxins and improving systemic hemodynamics. Whether such improvement could be translated into survival benefit is still uncertain, given the paucity of randomized controlled trials available. The outcome of patients receiving MARS treatment is difficult to analyze because liver failure patients constitute a heterogeneous population and different subgroups carry different prognoses. An evidence-based recommendation on the timing of MARS initiation is not available and currently MARS is usually commenced for hyperbilirubinemia or presence of complications of liver failure. MARS is in general a safe procedure, but there are still potential complications that need to be cautioned, along with various operative issues that are worth attention. The future prospects of MARS would rely on the completion of adequately powered randomized controlled trials [Chiu and Fan, 2006; Kamath, 2002; Rozga, 2006; Onodera et al., 2006].

Fractional plasma separation and adsorption (FPSA)
The Prometheus system is a FPSA method combined with high-flux hemodialysis (see Figure 4.4). FPSA uses an albumin permeable membrane with a cut-off of 250 kDa. Both albumin and albumin-bound toxins cross the membrane and pass through special adsorbers containing a neutral resin adsorber and anion exchanger. In a clinical application on 11 patients, Prometheus treatment significantly improved serum levels of conjugated bilirubin, bile acids, ammonia, cholinesterase, creatinine, urea, and blood pH [Onodera et al., 2006].

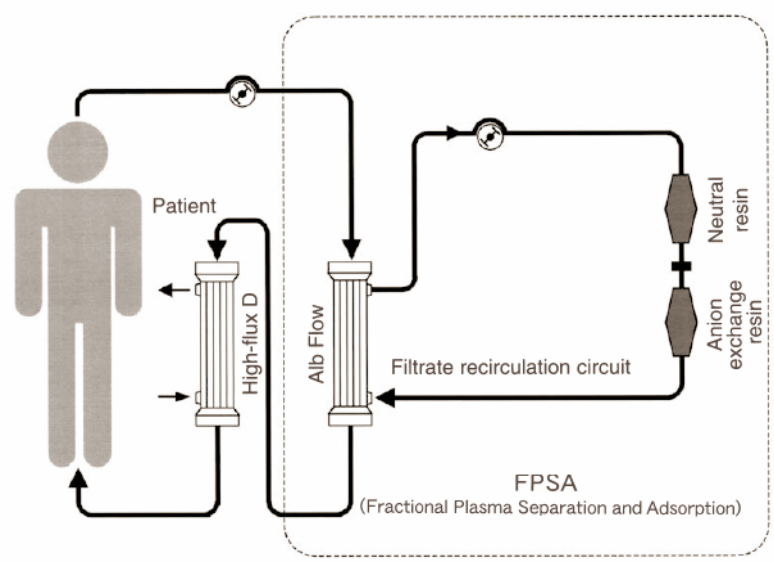

Figure 4.4. The circuit of the Prometheus system. Blood flows through a special albumin-permeable filter, AlbuFlow, in which the patient's own albumin (Alb) is separated from the blood. The albumin is then perfused through two adsorber cartridges. The purified albumin then reenters the blood stream. Afterwards the blood passes through a high-flux dialyzer. *(Source Onodera et al., 2006, courtesy of Springer, Heidelberg).*

Single-pass albumin dialysis (SPAD)
Adsorbents, such as charcoal and resins, are not very effective in removing proteins from the blood, including mediators of inflammation and inhibitors of hepatic regeneration. Therefore, MARS, Prometheus and other sorbent-based systems may have a limited therapeutic efficacy in patients with hepatic failure and hypercytokinemia, like an acute exacerbation (flare) of chronic hepatitis B infection. In this regard, single pass albumin dialysis (SPAD) appears to hold greater promise. SPAD is carried out using hollow fibers made of a highflux albumin-permeable membrane. Human albumin is added to the dialysis solution (4.4%) as a binding agent, to enable solute transfer from the patient's blood to the dialysis solution. Elimination of adsorbents and use of a standard dialysis hardware simplifies therapy and lowers its cost. Pre-clinical data and initial clinical experience with SPAD indicate that this simple, inexpensive technology merits further development and evaluation in appropriately designed trials [Rozga, 2006].

Selective plasma exchange therapy (SEPET)
As with SPAD, SEPET was designed as a blood filtration device that facilitates removal of the plasma fraction that contains mediators of inflammation and inhibitors of hepatic regeneration as well as direct hepato- and neuro-toxins. At the same time, important blood components that have a molecular weight greater than 100 kDa, including immunoglobulins, complement system proteins, blood clotting factors and HGF, are retained in the blood circulation. During SEPET therapy, which is provided using standard dialysis hardware, the removed toxic plasma fraction is replaced with electrolyte solution and a limited amount of human albumin and fresh frozen plasma [Rozga, 2006].

4.1.2 Applications in the Netherlands
The Health Council of the Netherlands reported in 2004 that the MARS system was promising [GR, 2004]. At that time it had been applied in the Netherlands on a couple of dozen patients. The Health Council concluded that albumin dialysis is able to stabilize the patient during the acute phase of liver failure, resulting in an improvement of the neurological

status (hepatic coma and intracranial pressure). The improved hemodynamic condition also results in recovery of the cerebral blood flow and renal function. Compared to standard treatment, MARS therapy may prevent acute mortality. This effect appears to be significant in patients with acute-on-chronic liver failure. The advantage seems less clear in patients with acute liver failure. The main application of MARS therapy is for the moment that of providing a bridge to transplantation. An important finding is that albumin dialysis with MARS has no significant adverse effects and can be tolerated for a longer period. The need for robust clinical studies remains, however, urgent.
Guidelines from the Dutch Society for Gastrology and Liver Specialists [NGMDL, 2003] and the Dutch Society for Internists [NIV, 2003] are less positive. They state that all liver support systems are in an experimental phase. 'High volume plasmapheresis' and MARS-therapy seem to be useful to support the detoxification capacity of failing liver, but offer no increase of the survival rate.
Personal communication (July 2007) with a liver specialist in one of the Dutch university hospitals learned that, to the knowledge of this person, in the Netherlands artificial liver devices are not used on patients with acute or acute on chronic liver failure. In the hospital where this physician is working, clinical studies are in the early stages of preparation to look into the effect of MARS therapy on cerebral blood flow in patient suffering from acute liver failure.

4.2 Cell/tissue-based approach for function recovery

Besides detoxification/purification, truly effective liver support systems should provide all liver functions, including biosynthesis and bioprocessing. Such systems can be achieved only with devices that bring the blood of the patient in contact with living liver cells: bioartificial liver systems (BAL). The cell activity can then contribute to the compensation of the failing patient liver, by e.g. detoxification, biosynthesis and biotransformation. Bioartificial livers come in various forms but generally consist of a cartridge (bioreactor) filled with living liver cells that is connected to the circulatory system of the patient. In this regard the use of a plasma recycling dialysis system which incorporates a liver cell-based bioreactor, may be a future ideal form of bio-artificial liver (BAL) support system [Nishimura et al., 2006].
BAL's can be classified according to the cell source, type of culture system / type of bioreactor. In this review the focus lies on the systems that are being used in clinical (trial) situations.

4.2.1 State of development

Cell source
The cell qualities that are looked for in a bioartificial liver are: safety, constancy of function and sustained proliferation. Various cell sources score differently for these qualities, and the optimal solution is not at hand yet.
Human hepatocytes can be used but have the drawback of a limited supply. Furthermore, when the cells are removed from the complex liver structure, expression of the complete spectrum of liver-specific functions becomes unstable in culture [Ambrosino and D'Amico, 2003]. Interaction between non-parenchymal cells and bile duct cells appears to be important for the optimal liver function.
An alternative is provided by immortalized human liver cells (by inactive retrovirus) or hepatoma cell lines, which have the capacity for sustained proliferation. However, being modified liver tumour cells they pose concerns of failing functional capacity and tumorigenic

risk [Onodera et al., 2006]. In order to reduce this risk of escape of tumourigenic cells into the patient various genetic engineering techniques (e.g. introducing suicide genes) have been studied.

Porcine cells are readily available and have been used extensively, fresh or cryopreserved. However, xenografts introduce specific immunologic risks (rejection) and warrant specific counter measures. Because of these immunological reactions treatment is advised not to extend to more than a week [Chamuleau, 2003] and this limited exposure is included in trial treatment regimes, e.g. 6 hours daily for a maximum of 14 days [Demetriou et al., 2007]. Furthermore, transmission of Porcine Endogenous Retro Virus (PERV) in vitro has been described. It is appreciated that there is a risk for transmission of PERV to humans, although this risk has not been substantiated in patients [Ambrosino and D'Amico, 2003; Onodera et al., 2006].

Alternative human cell sources are thought to be found in stem cells. Apart from e.g. embryonic or bone marrow stem cells, there are cells inside the liver that can contribute to liver (re-) generation. These intra-organ stem cells (found in e.g. the canals of Hering and intrahepatic bile ducts) become active in case of massive hepatic necrosis [Min and Theise, 2004].

Type of bioreactor
The conditions inside a bioartificial liver should stimulate long lasting cell function, provide scalability and stimulate mass exchange. To this end, various techniques for cell arrangement have been applied: monolayers, co-culture with nonparenchymal cells, fixation to a scaffold, rotation culture, etc [Onodera2006]. Furthermore, these cells should be protected from toxic influences from the plasma of hepatic insufficiency patients, e.g. by pre-treatment though an artificial system. In order to bring a sufficient amount of cells in contact with patient serum or blood various configurations have been studied: hollow fibre, flat plate monolayer, perfusion bed or scaffolds, suspension and encapsulation chambers [Onodera et al., 2006]. The serum or blood can be in contact with the cells directly or via a semi-permeable membrane.

Clinical trials:
Several approaches have been entered into clinical trials, aimed to function as a bridge to liver transplantation. This includes both human and porcine cell systems. A Cochrane Review of the clinical experience up to 2002 with artificial and bioartificial systems indicates that these support systems did neither influence mortality rates, nor bridging to transplantation, but improved neurological status [Liu et al., 2004]. Subgroup analysis revealed that mortality was improved in acute-on-chronic liver failure, but not in acute liver failure. However, based on the available evidence, no support system could be recommended for routine use.

<u>Human cells:</u>
As published by Sussman [Sussman, 1992], the Extracorporeal Liver Assist Device (ELAD, Vital Therapies, San Diego, USA) consists of a hollow-fibre device containing about 200 grams human hepatoblastoma-derived cells. Initial devices were perfused with blood. A randomized controlled trial in Acute Liver Failure (in 1996) failed to show clinical survival benefit. The current versions of the ELAD use four cell cartridges placed in a plasma re-circulation loop. Future clinical trials are to be expected in China [Rozga, 2006].

Porcine cells:
Demetriou et al. [2004] developed the HepatAssist BAL (Cedars-Sinai Medical Center, Los Angeles, USA), which accommodates 5-7 billion cryopreserved porcine hepatocytes. Hepatocytes are not grown in the reactor but are placed in the reactor shortly before application. An attachment surface is provided by collagen-coated dextran beads. A phase I study showed significant improvement in neurological condition and successful bridging to (not always successful) transplantation. The subsequent phase II/III study included a randomized, multicenter, controlled trial design. The study included 86 controls and 85 treated patients and did not show improvement of survival after 30 days. However, correcting for the impact of transplantation and for time to transplant, the HepatAssist BAL improved survival. For future trials improvements have been devised, like increasing the amount of cells, from 5-7 to 15-20 billion [Rozga, 2006].

The BioArtificial Liver from Amsterdam (AMC-BAL, the Netherlands) consists of a hollow-fibre-reactor with about 200 g (20 billion cells) porcine hepatocytes attached to a polyester matrix [Van de Kerkhove et al., 2002]. In between this matrix are hollow fibres for gas exchange. The patient serum is perfused through the cell compartment allowing direct contact with the hepatocytes. A clinical study showed that out of 12 patients all had alleviation of serum parameters and improved neurological state. Eleven were bridged to liver transplantation and one survived after BAL-treatment only; four patients died after liver transplantation [Van de Kerkhove et al., 2002].

Gerlach et al. [1994] developed the Modular Extracorporeal Liver Support (MELS, Charite Berlin, Germany), which consists of a bioreactor containing up to 650 g (about equal to the mass of a human liver lobe [Rozga, 2006]) hepatocytes combined with albumin dialysis (detoxification) and hemodiafiltration (removal of water soluble substances). Two clinical studies have been performed. One initial study with porcine cells described eight patients who were treated 8-46 hours bridging to transplantation [Vanholder et al., 2005]. They did well after 2 years and no antibodies against PERVs were detected. Another study with human cells is ongoing and includes 12 patients [Vanholder et al., 2005].

The Bioartificial Liver Support System (BLSS, Excorp Medical Inc., Minneapolis) [Stadlbauer and Jalan, 2007] contains approximately 100 grams of primary porcine hepatocytes and was used in a Phase I study in 4 patients and showed only a moderate biochemical response.

Other systems that use porcine hepatocyte cells have been reported to be tested in various clinical settings: TECA device from Beijing, China; a hybrid BAL from Ninjing, China; Radial Flow Bioreactor BAL from Ferrara, Italy. These systems contain 70-200 g of porcine cells. [Park and Lee, 2005, Rozga, 2006]. Furthermore, about 8 other BALs have been developed and are being studied in the pre-clinical phases [Park and Lee, 2005]. It is therefore to be expected that more BALs will be introduced in the clinical (trial) field in the near future.

Pre-clinical research developments:
Recombinant hepatic cell lines have been genetically modified to boost specific enzyme clearance pathways. These studies have not yet reached the clinical stage. For toxic substances like ammonia and diazepam these cell lines showed improved survival time in dogs with diazepam overdoses [Wang et al., 2005].

Furthermore, research continues on optimization of materials, e.g. Polyurethane spheroid culture system performed better in vitro when exposed to plasma of patients with fulminant hepatitis. The use of porcine or human cells was not different [Onodera et al., 2006].

Challenges:
In addition to the variable nature of the clinical effects of liver failure, differences exist in design of the bioreactors. Therefore, their clinical performance cannot readily be predicted based on parameters like cell mass, mass transfer rate, mode of oxygenation, treatment duration, direct/indirect contact between plasma and cells and flow/exchange rates [Park and Lee, 2005].
Based on the current clinical experiences, three problems have been mentioned in relation to the biological and physical limitations of bioreactor design [Vanholder et al., 2005; Pless and Sauer, 2005]:

- exchange capacity: normal blood flow through the liver is about 1.5 l/min, in a bioartificial device flow rates are limited to 0.1 to 0.3 l/min. because of technical and rheological reasons.
- cell mass: in living donation transplantation the transplanted cell mass should at least be 40% of the ideal liver mass of the recipient to meet the metabolic needs otherwise the graft may fail. Currently, cell mass in BALs ranges from 50-500 g, which is at most about one third of the weight of an adult liver.
- related to the problem of cell mass: a cell source that can supply large amounts of hepatocytes of good quality. Discarded livers for transplantation may serve to this end, but their number is limited (about 1 in 5 explanted organs) and their cell quality is not optimal due to preservation and isolation processes.

It is very clear that randomized controlled trials are mandatory to establish clinical efficacy, but it is less clear how the ideal trial should be constructed [O'Grady, 2006]. Furthermore, apart from desirable clinical results, the use of bio-artificial support systems can come with adverse events. In general the systems appear to be safe, but adverse events have been reported and the most important events are bleeding, systemic infection, disseminated intravascular coagulation, allergic shock, increase in intracranial pressure, hypotension and hypersensitivity [Stadlbauer and Jalan, 2007].
The causes leading to liver failure are various. In clinical studies focusing on liver failure (treated with bioartificial devices) it can be difficult to recruit a homogeneous and large enough group of patients. In case multicenter trials are conducted, care must be taken to harmonise the 'standard' level of care in the control group [Demetriou et al., 2004]. Furthermore, the contribution of the devices to the outcome can not be separated from the effect of subsequent liver transplantation. It may very well be that some subgroups of patients benefit from treatment with a bioartificial device, while others do not. It is suggested to focus on well defined groups (like liver failure due to alcohol) for which a liver transplantation can be contraindicated [Vanholder et al., 2005]. Other recommendations have been discussed and include: comprehensive understanding of the acute liver cell failure mechanisms, classification of conditions that require liver support, clear definition of treatment success [Adham, 2003].
From the clinical experiences of recent years and the adaptations in design of the newer BALs one can identify three trends, partly solving the abovementioned problems: 1) cell mass is increasing (towards 20 billion cells), 2) immunological barriers are not a requirement (short term contact with xenogenic cells does not induce important immunological complications), 3) hepatocytes are being cultured as organoids ,i.e. having appropriate cell-cell interactions, including interaction with non-parenchymal cells [Park and Lee, 2005].

Given the fact that the majority of BALs use porcine cells, it can be expected that future clinical trials will be conducted in countries that do not pose regulatory objections against clinical use of xenogenic 'devices'.

4.2.2 Applications in the Netherlands

The Health Council of the Netherlands reported in 2004 that the bioartificial systems for liver support (BAL) are still experimental and few clinical results are available [GR, 2004]. The Health Council focussed their report on BAL's with animal cells. The BAL may be developed as a bridge to transplant for patients suffering from acute-on-chronic liver failure or for some patients as a bridge to recovery. Concern exists about the possible adverse immunological reactions towards the cells in the BAL, the transfer of cells into the patient which may lead to tumors and the transfer of retro viruses to the patient that may eventually pose a public health hazard. The further research of BAL devices incorporating porcine cells is banned in many countries around the globe; also in the Netherlands. This is a considerable drawback for a thorough evaluation of the efficacy and safety of the BAL [GR, 2004].

4.3 Conclusion

Enthusiasm for liver support devices, particularly cell-based biological systems and albumin dialysis, has increased over the last decade resulting in considerable clinical activity both within and without the construct of clinical trials. Most data have been generated on patients with acute liver failure or in patients with decompensation of chronic liver disease, often referred to as acute-on-chronic liver failure. In clinical use for acute liver failure, bridging to liver transplantation is a more realistic goal rather than to transplant-free survival. In acute-on-chronic liver failure the objective of attaining clinical stability with treatment appears more achievable.

Currently, there is no single artificial organ or device capable of emulating all the functions of the liver. Some functions related to removal of toxic substances can be emulated by liver dialysis, charcoal hemoperfusion or plasma exchange, experimental treatments for liver failure. These methods have not yet been shown to improve the survival of patients with liver failure, although hemodialysis did work well on renal failure associated with liver failure. A small clinical trial (n=5) using a combination of slow plasma exchange in combination with high-flow continuous hemodiafiltration showed some promise. The most promising medical device approaches at this moment are SPAD (single pass albumin dialysis) and MARS (molecular adsorbent recycling system), which combines conventional dialysis with albumin dialysis. Both approaches are still in an experimental phase and the future prospects rely on the performance of adequately powered randomized controlled trials. In the Netherlands artificial liver devices are currently not used on patients with acute or acute on chronic liver failure, although early stages of clinical studies on MARS therapy are being explored.

Medical devices based artificial liver support systems have a beneficial influence on the neurological state of patients, but do not improve survival. More beneficial effects have been expected from systems that bring the blood of the patient in contact with living liver cells, or: bioartificial liver systems (BAL). The cell activity can then contribute to the compensation of the failing patient liver, by e.g. detoxification, biosynthesis and biotransformation. The BAL may be developed as a bridge to transplant for patients suffering from acute-on-chronic liver failure or for some patients as a bridge to recovery.

The BAL based on porcine hepatocytes, is the most extensively evaluated type of biological device. A sizeable clinical trial failed to demonstrate efficacy, but secondary analyses suggest

it would be unwise to assume futility had been established with this device. Concern exists about the possible adverse immunological reactions towards the animal cells in the BAL, the transfer of cells into the patient which may lead to tumors and the transfer of retroviruses to the patient that may eventually pose a public health hazard. The further research of BAL devices incorporating porcine cells is banned in the Netherlands as well as in many other countries.

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5. Kidneys

While the kidney is mostly seen just as an organ of excretion, it is more than that. It does remove wastes (urea, ammonia, drugs, toxic substances), but it also removes normal components of the blood that are present in greater-than-normal concentrations. When excess water, sodium ions, calcium ions, potassium ions, and so on are present, the excess quickly passes out in the urine. On the other hand, the kidneys step up their reclamation of these same substances when they are present in the blood in less-than-normal amounts. Thus the kidney continuously regulates the chemical composition of the blood within narrow limits. The kidney is one of the major homeostatic devices of the body. The kidney helps to regulate the blood pressure and stimulates the making of red blood cells. The human kidney is also an endocrine gland secreting the hormones erythropoietin and calcitriol, the active form of vitamin D, as well as the enzyme renin.
The basic unit of the kidney is the nephron (see Figure 5.1). It is a long thin tube that is closed at one end, has two twisted regions interspaced with a long hair-pin loop, ends in a long straight portion and is surrounded by capillaries. In each kidney, there are one million nephrons.

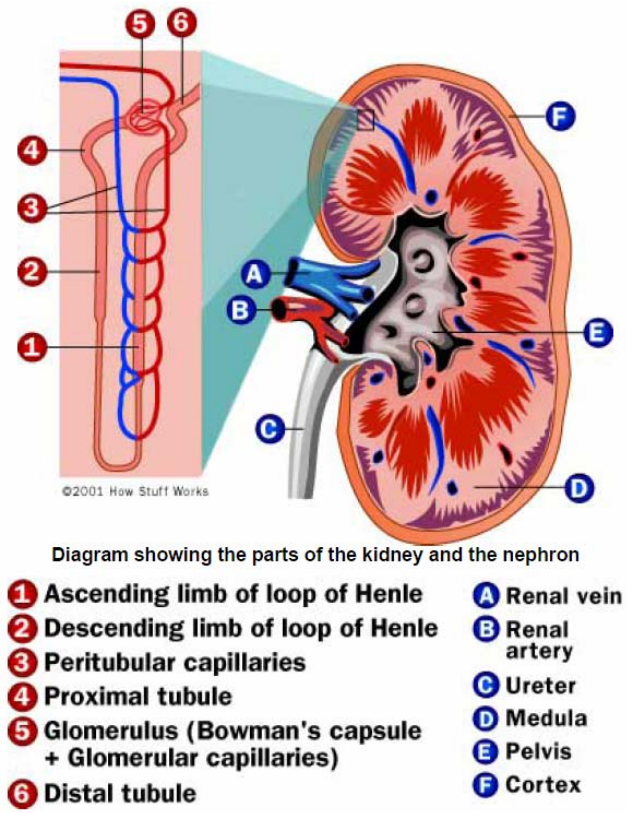

Figure 5.1 Diagram showing the parts of the kidney and the nephron
(Source: Freudenrich on http://www.Howstuffworks.com)

In the nephron, approximately 20% of the blood gets filtered under pressure through the walls of the glomerular capillaries and Bowman's capsule. The filtrate is composed of water, ions (e.g. sodium, potassium, chloride), glucose and small proteins (less than 30,000 daltons). The rate of filtration is approximately 125 ml/min or 180 litres each day. Also, the amount of any

substance that gets filtered is the product of the concentration of that substance in the blood and the rate of filtration. The arrangement of the glomerular capillaries in series with the peritubular capillaries is important to maintain a constant pressure in the glomerular capillaries, and thus a constant rate of filtration, despite momentary fluctuations in blood pressure. Once the filtrate has entered the Bowman's capsule, it flows through the lumen of the nephron into the proximal tubule. The nephron reabsorbs the components that the body needs from the lumen back into the blood. Once inside the lumen of the nephron, small molecules, such as ions, glucose and amino acids, get reabsorbed from the filtrate. Specialized proteins called transporters are located on the membranes of the various cells of the nephron. Reabsorption of most substances is related to the reabsorption of sodium, either directly, via sharing a transporter, or indirectly via solvent drag, which is set up by the reabsorption of sodium. The nephron secretes some unwanted components from the blood into its lumen, eg ammonia. As in reabsorption, there are transporters on the cells that can move these specific substances into the lumen. Anything (fluid, ions, small molecules) that has not been reabsorbed from the lumen gets swept away to form the urine, which ultimately leaves the body. Through these processes, the blood is maintained with the proper composition, and excess or unwanted substances are removed from the blood into the urine [Freudenrich, 2006].
End stage renal disease (ESDR) is treated with different strategies. The best survival outcomes may be expected by live donor renal transplantation. The mortality rate of patients with renal transplants is approximately half that of similar patients remaining on dialysis, despite improvements in dialysis and the morbidity of transplant surgery and immunosuppressive drugs. Transplantation is, however, severely limited by the supply of donor organs; more than three-quarters of transplant candidates die waiting for a kidney.

5.1 Medical device-based approach for function recovery

One of the oldest technologies developed to support or take over functions of a failing organ is dialysis. Although this treatment lays a heavy burden on the patient and comes with considerable side effects, it is currently still the standard therapy for end stage renal disease. The technology effectively serves as a bridge to transplant. More recently, research is aiming to develop devices which can replace other functions of the kidney besides the clearance of small “chemical waste” molecules from the blood stream. The ultimate aim is to construct an implantable artificial kidney.

5.1.1 State of development

Dialysis

The natural kidney provides its homeostatic and clearance function continuously within the body. Current dialysis techniques provide around 10% of the clearance power of the natural kidney. Hemodialysis (HD) is a technique in which blood is continuously extracted from the patient and directed over a membrane which is in contact with an electrolyte solution known as dialysate. The membrane is designed to allow for diffusive transport of small molecules from the blood to the dialysate while retaining the higher molecular weight compounds such as albumin, coagulation proteins and immunoglobulines in the bloodstream. Dialysis is performed periodically, typically 3 sessions per week, 4-5 hours per session. The short and infrequent dialysis is associated with an increased risk of under dialysis, fluid overload and hypertension, all factors associated with increased mortality and morbidity. The fluctuations in fluid state make it very difficult to control blood pressure and volume over the weekly

cycle. Phosphate control is impossible without strict compliance with a phosphate binding or restriction regime.
Conversely, longer dialysis periods (8-12h) have been shown to maintain blood pressure within the normal range without drugs and to improve survival. Daily dialysis has been shown to normalize blood phosphate without the need for phosphate binders in addition to improved blood pressure control. Longer or more frequent dialysis strategies have not been generally accepted as they are considered to be impractical in the hospital setting. However, if the dialysis is delivered at home (or nursing home), longer and more frequent regimes become practical. At present, home dialysis is available only for a limited number of patients [Hollestelle et al., 2005]. The technology needs to be developed further to make it really practical and convenient for routine home use for the typical dialysis patient.
Modern dialysis is carried out with dialysers with minimal blood priming required, constant and reproducible performance and minimal dialytic loss of essential constituents such as albumin, despite significantly greater clearance characteristics for small- and medium-sized solutes compared to previously available dialyzers. Further increases in dialyser efficiency are increasingly difficult, in large part because of the non-selective nature of current conventional membranes, except on the basis of solute molecular size.
Another problem with HD is the vascular access. The typical bloodflow of 400-500 ml/min requires a specialized vascular conduit known as a fistula if made from native vascular tissue, or known as a graft if constructed from synthetic material. Vascular access failure is common; 49% at 18 months for arteriovenous fistulas and 67% for grafts.
Alternatively, dialysis can be effectively performed inside the patient's own abdominal cavity using the peritoneal epithelia as the dialysis membrane; peritoneal dialysis (PD). PD is typically performed at home by the patient themselves, permitting the patient to remain in school, work and enjoy considerable independence, when compared with three-times weekly, in-centre dialysis. PD is, however, not possible in patients with previous abdominal surgery, hernias or peritoneal scarring, and despite significant advances in the connectors used in peritoneal dialysis, peritonitis remains quite common and may prevent continuing treatment.
Both PD and HD show similar survival benefit in ESRD, with a strong age effect on survival. Teenagers have a 90% 5-year survival on dialysis. This drops to 53% by the age between 40 and 50 and is below 20% by the age of 70. Five-year survival is improved by a factor of four for elderly recipients of a living donor transplant, when compared with patients remaining on dialysis.

Towards the implantable artificial kidney
The research in renal replacement therapy (RRT) must expand from optimizing a chemical purification technique applied to reproducing the function of a complex multicellular organ. The ideal RRT device would mimic the function of natural kidneys – it would be continuously operating, remove solutes with a molecular weight spectrum like natural kidneys, it would be flexible and remove water and solutes based on individual patient needs, it would be wearable or implantable, and it would be biocompatible. In addition, it would be lightweight, low cost, safe and reliable (see Figure 5.2) [Tattersall, 2001].
Dialysis provides clearance of small molecules in blood by diffusive flows across a semipermeable membrane and control of volume status by bulk flow of water and solutes through that membrane. These effects are sufficient to counter the lethal acidosis, volume overload and uremic syndromes which accompany renal failure. The metabolic, endocrine and immune functions of the healthy kidney are not performed by either HD or PD; explaining the low survival of patients who depend on dialysis.

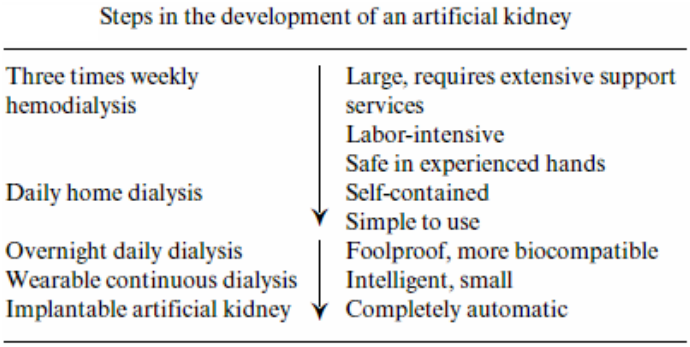

Figure 5.2 Flow scheme showing steps in the development of an artificial kidney.
(Source: Tattersall, 2001)

There are two approaches to engineering tubular reabsorption: employing living cells to mimic the function of their native counterparts (see section 5.2), or manufacturing a second filtration membrane which permits the passage of salt, water, glucose and sodium bicarbonate, but retards the passage of uremic toxins. The advantage of the former lies in the simplicity of the approach; there is no need to separately implement each of the many transporters on the apical surface of the cell; supply the cell and the cell will supply not only the transporters but in addition, the driving force for reabsorption.

Renal replacement therapy (RRT) using smart nano-membranes
Nissenson and colleagues [2005] have proposed a novel renal replacement device, called the Human Nephron Filter (HNF). The HNF consists of two membranes operating in series within one device cartridge. The first membrane, the G membrane, mimics the function of the glomerulus, using convective transport to generate a plasma ultra filtrate containing all solutes approaching the molecular weight of albumin. The second membrane is the T membrane, the smart membrane, which mimics the function of the renal tubules, selectively reclaiming designated solutes to maintain body homeostasis, again by convection. No dialysate is used in this system. The cartridge with the membranes is part of a wearable system that includes a keypad and display, a high-capacity battery, and a waste bag. The entire device is wearable, slightly larger than a natural human kidney, and weighs about 3.2 kg in its initial version. Once blood access is obtained, blood is pumped at 100 ml/min across the G membrane. The total surface area need is just over one hundredth of a square meter. The membrane consists of approximately 1.6×10^{16} pores, 1–5 nm apart. Pores can be constructed in various sizes and shapes. Although the initial T membranes will be developed with only one type of pore, future plans include the development of a pore library that will permit custom membranes to be produced, depending on patient needs.
Some key differences between smart membranes and conventional polymer membranes are noted. Smart membranes have selective transport characteristics, contain a predetermined number and size of pores that are atomically engineered and are not passive, but have specific interaction with solutes. In addition, the active filtration layer is ultra-thin, the size of a single molecule (about 2.5 nm). In contrast, polymer membranes have unselective transport of solutes, a wide distribution of pore sizes, the distribution of which is not easily controlled, and these membranes are thick [Nissenson et al., 2005].
When evaluating the interactions of pores with solutes and solvents at the atomic level, both the shape of the solvent sphere around the solute as it approaches the pore and the tightness with which the solvent molecules are bound to the solute will determine the amount of energy required to allow the solute to move through a particular pore. Pores have been developed such that the energy requirements to dehydrate ions are so great that they cannot be met, and restriction of ion movement through the pore occurs. Pores can be developed with similar

radii, but with dramatically different and selective solute passage characteristics. Modeling studies have been carried out studying the performance of the HNF for urea, β_2-microglobulin, and a variety of other solutes. Simulating 30 ml/min glomerular filtration rate would result in a time-averaged urea concentration (TAC) in the modeled patient of about 27 mg/dl, with minimal fluctuations of blood urea nitrogen throughout a weekly cycle. By contrast, the thrice-weekly dialysis simulation yields a TAC of 67.3 mg/ml, with wide excursions of blood urea nitrogen reflecting the intermittent nature of the treatment. If the HNF were to run continuously, the TAC urea would fall to normal levels. With 12-hour, 7-days/week treatment, levels of β_2-microglobulin are predicted to approach normal. The removal of key substances including sodium, potassium, calcium, magnesium, phosphorus, and bicarbonate is considered in the model. In a 70-kg patient on a normal diet, the initial HNF system is capable of maintaining balance for all of these substances except bicarbonate, which may need additional supplementation [Nissenson, 2005]. Similar research and developments in Japan were described by Saito, also giving promising results [Saito, 2005]. An extended version of HNF is described by Jennings on his website. His Implantable Human Kidney Replacement Unit (IHKRU) contains 20 membranes, 12 reverse osmosis membranes for ultra-filtration, and 8 osmosis membranes for re-absorption. The membranes are all designed to filter molecules within a specific dimensional range. The available description of the device is limited and purely theoretical. Jennings does not give any indication whether this device is actually under development. It is not referenced in literature, but patents are submitted. [http://implantablekidney.com/] Van Geertruyden announced the development of ceramic membranes that will both have a smaller pore size distribution as well as an improved filtration rate, when compared to traditional filters [http://www.physorg.com/news94407326.html].

5.1.2 Applications in the Netherlands

Since 2006, the Dutch Kidney Foundation (DKF) [www.nierstichting.nl] participates in the BioMedical Materials Program (BMM) [www.biomedicalmaterialsprogram.nl]. BMM is a consortium of leading Dutch companies, small and medium sized companies, knowledge institutes and public organizations. Within BMM, DKF stimulates the development of an implantable artificial kidney, to be available within ten to fifteen years. The aim for the first five years is to decide which of the two approaches, nano-membranes or the bioartificial kidney has the most potential to be developed into a working implantable artificial kidney. University lecturer Rabelink expects the first nano-filter based artificial kidney to be available in five to eight years [Marx, 2007].

5.2 Cell/tissue-based approach for function recovery

Long-term chronic renal replacement therapy with hemodialysis and peritoneal dialysis provides mostly intermittent filtration function and continues to have unacceptably high mortality and morbidity rates. This is mainly caused by the fact that these approaches lack the potential to fully mimic the main metabolic functions of the kidney, such as i) removal of toxic waste products, ii) regulation of electrolyte balance, iii) removal of excess water, iv) calcium and phosphate metabolism, v) erythropoietin production. In Figure 5.3, an overview is provided of the symptoms that arise when these main functions are insufficiently replaced or restored using a conventional therapy, including the underlying biological mechanisms.

Kidney function	Conventional therapy	Symptoms if inadequate	Main biological mechanism
Removal of toxic waste products (uremic toxins).	Hemodialysis, hemodiafiltration and peritoneal dialysis.	Chronic inflammation, polyneuropathy, amyloïdosis.	Partial clearance by glomerular filtration. Active secretion or breakdown by proximal tubule cells.
Regulation of electrolyte balance.	Hemodialysis, hemodiafiltration and peritoneal dialysis.	Hemodynamic instability, edema.	Complex interactions involving active and passive transport mechsnisms in the proximal and distal part of the tubules.
Removal of excess water.	Ultrafiltration.	Hemodynamic instability, edema.	Hormone regulated watercahnnels in the distal tubules and collecting duct cells.
Calcium and phosphate metabolism.	Dietary restrictions, calcium supplements, phosphate binders.	Hypocalcaemia, hyperphospatemia, osteodystrophy, secondary hyperparathyroiditis.	Vitamin D3 production in the proximal tubule cells. Resorption of calcium and phosphate in the proximal tubule cells.
Erythropoietin production.	Daily injections of erythropoietin.	Anemia, fatigue.	Production by peritubular cells.

Figure 5.3 Overview of shortcomings of conventional therapy *[Boomker and Van Luyn, 2006]*.

This illustrates that for all of these functions specific cellular responses are required, which are known to be part of complicated regulation loops. Cell-based artificial kidneys that replace the kidney and cell therapies that enhance the natural repair mechanism of the kidney potentially include these metabolic functions of the kidney. Although replacing or repairing the full metabolic function seems to be a long-term goal due to the complex nature of the kidney, simple cell systems can already contribute to the replacement or repair of some kidney functions.

5.2.1 State of development

The current decade has witnessed the development of several cell-based approaches for kidney treatment, which can roughly be divided into the following categories:

I) Repair of the kidney by infusion of stem cells
II) Transplantation of fetal kidney tissue
III) Use of extracorporeal cell-coated devices
IV) Use of in vivo renal cell-coated matrices

I) Repair of the kidney by infusion of stem cells

The aim of this approach is to introduce supplementary cells into a damaged kidney to aid repair and regeneration. The most reported cell types used for this approach are bone marrow derived stem cells, which are easily accessible and can be expanded in vitro before reintroducing them as a therapeutic agent. However, there appear to be contradictory opinions regarding the function of bone marrow stem cells in renal repair. Some indicate that bone marrow-derived cells significantly contribute to daily turnover of renal tissue and to a high degree during recovery from tissue damage, whereas others indicate that this is a rare event, seen only to a very limited extent following injury [Brodie and Humes, 2005]. Poulsom et al. [2001] examined kidney biopsies from male patients who had received transplants from female donors, as well as female mice that had received a male bone marrow transplant. In both cases, they identified Y-chromosome-containing (and therefore host or donor-derived) cells within both tubules and glomeruli. The authors reported that 3.8 to 7.9% of cortical

tubular epithelial cells in the female recipients, 13 weeks following male bone marrow transplantation, contained a Y-chromosome. Although recipients were irradiated prior to bone marrow transfer, the authors note that nephric damage induced by this treatment is minimal, and they considered therefore that this figure represents a basal turnover level of contribution by circulating stem cells to the renal parenchyma. In addition, Kale et al. [2003] showed in an animal model that stem cell transplantation ameliorated the effects of ischemic injury, as blood urea nitrogen levels in the group that received transplantation were indistinguishable from unirradiated controls and lower than those of animals that did not receive stem cells over a 7-day period following injury.
To the contrary, several authors have reported that, although it is possible for bone marrow-derived cells to adopt proximal tubule cell fate, this is an extremely rare event [Szczypka et al., 2005; Gupta et al., 2002; Krause et al., 2001]. This lack of an emerging consensus during the expansion of this field has decreased the initial optimism over results that appeared to indicate a short journey to the clinic for transdifferentiating bone marrow stem cells.
Besides bone marrow derived stem cells, also renal stem cells have been reported to exist. Oliver et al. [2002; 2004] have identified a population of slow-cycling cells residing in the papilla of the adult kidney that proliferate in response to ischemia/reperfusion injury and may migrate to the medulla. In vitro, these cells were shown to have the potential to generate several epithelial elements of the nephron. The existence of such a cell type would have an immediate impact on the development of cell therapies for kidney disease. These cells could be isolated, culture expanded and infused into the patient. Nevertheless, the use of renal stem cells is still in an early research phase and far from clinical application. Before cell therapies that enhance the repair of the kidney can be introduced in the clinic, a clear understanding of the natural repair mechanism is required.

II) Transplantation of fetal kidney tissue
Another approach is the use of fetal tissue for the repair and replacement of renal function. Hammerman [2003] has shown that E15 rat metanephroi (the third stage of kidney development forming the adult kidney) transplanted into the omentum (a folded membrane within the abdominal cavity attached to the bottom edge of the stomach and the transverse colon) of adult host rats grow to approximately one-third the diameter of native kidneys, develop mature glomeruli and tubules, and are vascularized by arteries originating from the omentum. Inulin clearance in bilaterally nephrectomized hosts, following connection of the ureters of the implanted metanephros with the native kidney, has been measured at approximately 0.2% of the mean per single kidney from normal rats expressed as microliters per minute per 100g of rat weight and 9% of those measured per single kidney of normal rats expressed as microliters per minute per gram of kidney weight. Also the use of human embryonic metanephroi cells transplanted in mice has been explored, which revealed renal development and proper vascularisation [Dekel et al., 2002, 2003]. Should it prove possible through growth factor treatments and/or optimization of transplantation methodology to increase the functionality of transplanted metanephroi to physiologically significant levels, this approach may become clinically useful. Another approach by which embryonic stem cells were used to regenerate renal tissue has been reported by Kobayashi et al. [2005]. They have demonstrated that Wnt4-transformed mouse embryonic stem cells injected into an adult kidney developed tubule-like structures. Yamamoto et al. [2006] reported that embryonic stem cells injected into the kidney developed into an urereteral bud-like structure and expressed many genes that are expressed in developing kidneys. From these results, it is suggested that embryonic stem cells are a good candidate for kidney regeneration, although major ethical and immunological problems have yet to be overcome.

III) Use of extracorporeal cell-coated devices

A novel and very promising approach to renal replacement therapy relies on combining ultrafiltration by a conventional hemofilter with tubular reabsorbtion by a bioreactor of cultured kidney cells [www.nephrotherapeutics.com]. A tissue-engineered bioartificial bioreactor has been build by Dr. Humes and has entered clinical trials in the USA. The bioreactor, known as the renal assist device (RAD), consists of a confluent layer of cultured proximal tubule cells seeded on the lumen side of multiple polysulfone hollow-fibers (see Figure 5.4). The human kidney cells are isolated from organs donated for cadaveric transplantation, which could not be used for conventional transplantation due to anatomic or fibrotic defects. Humes' group has explored the metabolic characteristics of cultured proximal tubule cells by expressing the transport of glucose, bicarbonate and glutathione in terms of fractional reabsorption. For each of these molecules, fractional excretion was measured in the absence and presence of a known inhibitor of an enzyme essential for reabsorption. In each case, there was evidence of active transport and specific inhibition. Furthermore, the proximal tubule cells in the bioreactor have been demonstrated to stepwise increase ammonia production with changes in pH, which is essential for renal excretion of an acid load as it buffers secreted protons.

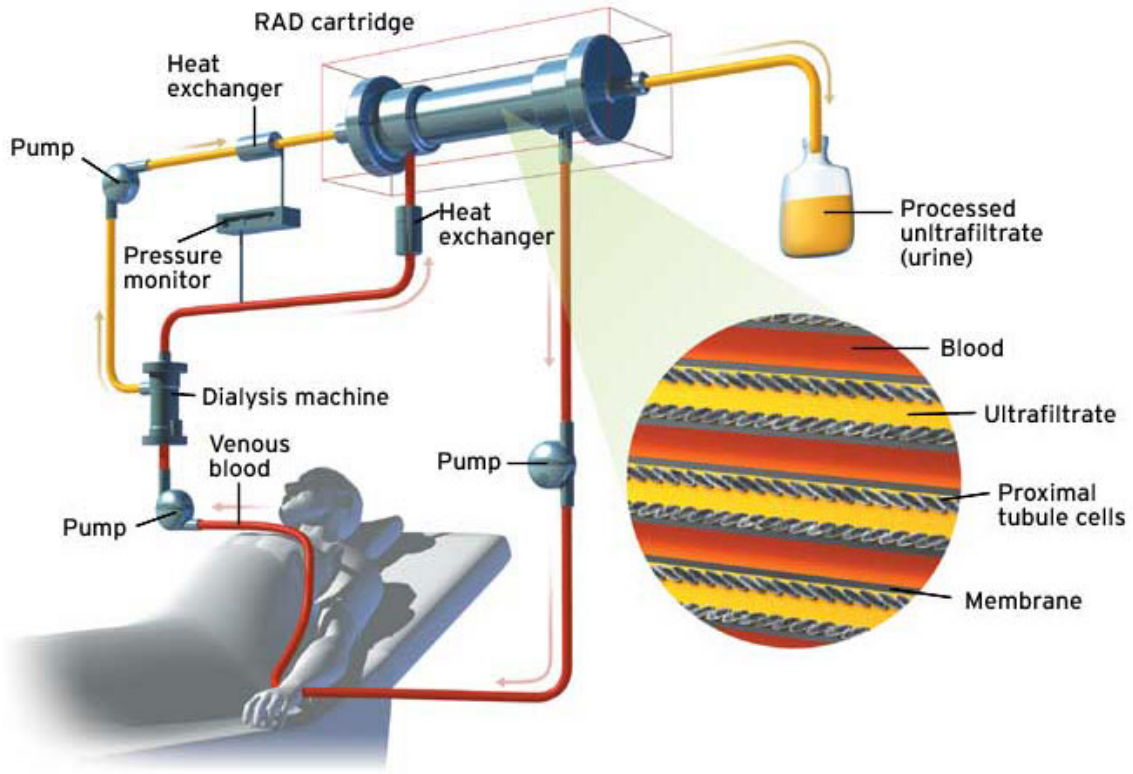

Figure 5.4 The renal assist device (RAD), under development at RenaMed Biologics *(Reproduced with permission of Bryan Christie Design).*

After the RAD is coupled to a filtration device (a conventional hemofilter) and connected to the patient, blood is pumped out of the body using a peristaltic pump. The blood then enters the hemofilter, where ultrafiltrate is formed and delivered into the tubule lumens within the RAD downstream of the hemofilter. Processed ultrafiltrate exiting the RAD is collected and discarded as urine. The filtered blood exiting the hemofilter enters the RAD through the extracapillary space port and disperses among the fibers of the device. Upon exiting the RAD, the processed blood travels through a third pump and is delivered back to the animal. According to the manufacturer, these cells: 1) recover essential non-waste blood products

from the passing filtrate and returns them to the circulation, 2) produce vitamins or vitamin precursors and release them into the circulation, 3) produce several soluble molecules critical for volume control and blood pressure regulation, 4) are the gatekeepers for residual toxic compounds in the filtrate, 5) sense infectious and inflammatory components and communicate with the body's host defense system.
Large animal studies have been completed with the use of this extracorporeal circuit. The RADs maintained viability and functionality in the extracorporeal circuit. During a 24-h perfusion period, fewer than 10^5 cells were lost from the RAD, which contained more than 10^9 cells. The experiments clearly showed that the combination of a synthetic hemofilter cartridge and a RAD in an extracorporeal circuit successfully replaced filtration, transport and some major metabolic and endocrinological functions of the kidney in acutely uremic dogs. After this series of experiments demonstrating bioactivity, longevity and systemic activity of the proximal tubule cells in a large animal model, experiments were conducted designed to examine the impact of cell therapy on the course of sepsis complicated by renal failure. This controlled trial of cell therapy of renal failure in a realistic animal model of sepsis revealed evidence that cell therapy with renal proximal tubule cells altered the physiological response to sepsis. Clear differences in survival and the serum cytokine associated with mortality in sepsis were found between animals treated with cells and with sham cartridges. This indicates that the increased mortality in renal failure has not been conclusively attributed to inadequate clearance, but may arise from other bioactivity of the kidney.
Furthermore, an FDA-approved phase I/II clinical trial on 10 patients has been completed. All 10 patients had acute renal failure and multi-organ failure, with predicted hospital mortality rates averaging above 85%. The results indicate that the RAD maintains viability, durability, and functionality in this ex vivo clinical setting. The device also demonstrated differentiated metabolic and endocrinologic activity, with glutathione degradation and endocrinologic conversion of 25-OH-D(3) to 1,25-(OH)(2)-D(3). All but one treated patient with more than a 3-day follow-up in the intensive care unit showed improvement as assessed by several physiologic parameters following therapy. Six of the 10 treated patients survived past 30 days. One patient died within 12 hours after RAD treatment due to his family's request to withdraw ventilatory life support. Three other patients died due to complications from acute or chronic co-morbidities unrelated to acute renal failure or RAD therapy. Plasma cytokine levels suggest that RAD therapy produced dynamic and individualized responses in patients. For the subset of patients who had excessive pro-inflammatory levels, RAD treatment resulted in significant declines in granulocyte colony stimulating factor (G-CSF), interleukin (IL)-6, IL-10, and IL-6/IL-10 ratios. In conclusion, the addition of human renal tubule cell therapy to dialysis treatment had been accomplished and demonstrates metabolic activity with systemic effects in patients with acute renal failure and multiorgan failure [Humes et al., 2004]. Currently, a Phase II clinical trial is ongoing of which an interim analysis has been reported [Tumlin et al., 2005]. In total, 58 patients at two medical centres were enrolled, including patients with dialysis-dependent acute renal failure receiving renal replacement therapy, and excluding patients with irreversible neurological damage or contraindication to systemic anticoagulation. The main outcome measure was 28-day all-cause mortality, which was measured at 34.3% in the treatment arm versus 55.6% for conventional treatment. This represents an approximate 20% absolute risk reduction and a 40% relative risk reduction for mortality. In the next 5 years, Phase III clinical trials of the technology will be completed in acute and chronic renal failure, and cell therapy of acute renal failure will likely be a mature technology available in multiple tertiary care centres [Fissell, 2006].

IV) In vivo renal cell-coated matrixes

The use of cell-coated implants is based on in vitro manipulation of cells of interest and their association with biomaterials, which may be either biodegradable or permanent in nature. The applied cells may either be of a specific cell type in order to replace a specific metabolic or catabolic function, or may present several cell types in order to substitute multiple functions of the kidney. Saito et al. [2003] have demonstrated the feasibility of using a cellular implant for continuous degradation of low molecular weight proteins such as β2-microglobulin (β2M). Working in nude mice, they first implanted a collagen sponge impregnated with basic fibroblast growth factor to promote vascularization and subsequently introduced megalin-expressing cells into the newly vascularized structure. The cells (L2), derived from a rat yolk sac carcinoma, were shown to take up and degrade circulating β2M, leading to a 40% reduction in the steady-state level of this uremic toxin in nephrectomized animals. However, the use of a tumor cell line in such a setting is obviously a clinical impossibility. Besides cell lines, the use of adult rabbit renal cortex cells seeded onto biodegradable polyglycolic acid sheets were reported to be extensively vascularized with the formation of glomeruli and organized tubule-like structures after subcutaneous implantation. It was, however, not specified whether these structures were interconnecting [Amiel et al., 2000].

Another interesting novel approach has been reported by Lanza et al. [2002] in which cell nuclear transfer (therapeutic cloning) has been used. Nuclear transfer enables the generation of cells genetically identical to an adult by the transfer of a somatic cell nucleus (in this case from a skin fibroblast) into an enucleated oocyte to generate a cloned embryo. The cells of this cloned metanephros (bovine) were dissociated, expanded in culture and seeded on scaffolds consisting of three collagen-coated cylindrical polycarbonate membranes (see Figure 5.5). The ends of the three membranes of each scaffold were connected to catheters terminating in a collecting reservoir. This created a renal neo-organ with a mechanism for collecting the excreted urinary fluid. Scaffolds with the collecting devices were transplanted subcutaneously into the same steer from which the genetic material originated and retrieved 12 weeks after implantation. Chemical analysis of the urine-like fluid (for urea nitrogen/creatinine levels, electrolyte levels, specific gravity and glucose concentration) revealed that the implanted renal cells possessed filtration, reabsorption, and secretory capabilities. Histological examination of the retrieved implants revealed extensive vascularization and self-organization of the cells into glomeruli- and tubule-like structures. A clear continuity between glomeruli, tubules, and the polycarbonate membrane was noted that allowed the passage of urine into the collecting reservoir. Immunohistochemical analysis with kidney-specific antibodies, RT-PCR analyses and western blot analysis revealed the presence and expression of renal specific proteins. In addition, it was demonstrated by using delayed-type hypersensitivity testing *in vivo* and Elispot analysis of interferon-gamma secreting T-cells *in vitro* that the oocyte-derived mtDNA was no source of immunologic incompatibility in the cloned renal cells. This study demonstrates that cells derived from nuclear transfer can be successfully harvested, expanded in culture, and transplanted *in vivo* with the use of biodegradable scaffolds on which the single suspended cells can organize into tissue structures that are genetically identical to that of the host [Hipp and Atala, 2004]. The acceptability of nuclear transfer as a method of generating non-immunogenic cells for the treatment of a particular individual is an issue that needs a thorough ethical debate. In the meantime, the approaches that protect cells from the host and vice versa by incorporating immunoisolation barriers (such as the RAD, described above) are most likely to advance the treatment of renal failure.

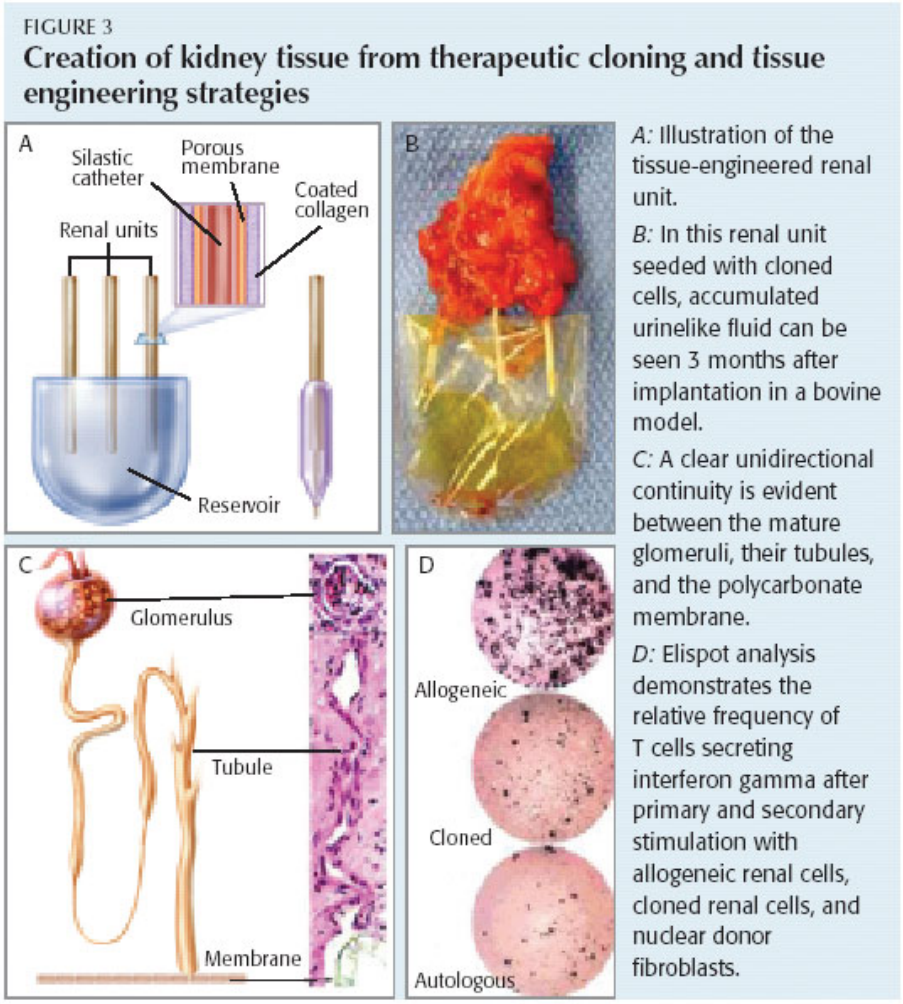

Figure 5.5 Creation of kidney tissue by combining therapeutic cloning and tissue engineering technologies
(Reproduced from Anthony Atala, M.D., Wake Forest Institute for Regenerative Medicine).

5.2.2 Applications in the Netherlands

Since 2006, the Dutch Kidney Foundation (DKF) [www.nierstichting.nl] participates in the BioMedical Materials Program (BMM) [www.biomedicalmaterialsprogram.nl]. BMM is a consortium of leading Dutch companies, small and medium sized companies, knowledge institutes and public organizations. Within BMM, DKF stimulates the development of an implantable artificial kidney, to be available within ten to fifteen years. The aim for the first five years is to decide which of the two approaches, nano membranes or the bioartificial kidney, has the most potential to be developed into a working implantable artificial kidney. Because a bioartificial kidney needs to perform different metabolic, endocrine and homeostatic functions, its design and construction is anticipated to be highly complex and will demand an interdisciplinary approach. The DKF aims to found a consortium of research groups that will collaborate in the construction of an implantable kidney. Dr. J. Boomker of the University Medical Center Groningen (UMCG) is playing a leading role and has made an inventory of the worldwide initiatives in the field of artificial kidneys and the state of the art in the Netherlands. Based on both the inventory report of Boomker and personal research and communication with research groups, the following summary of research in the Netherlands related to the development of cell based approaches to repair or replace the kidney is obtained.

At the Department of Nephrology, University of Nijmegen, a conditionally immortalized visceral epithelial cell line has been established. The cells were obtained from glomuli from H-2Kb-tsA58 transgenic mice that contain a gene encoding a temperature-sensitive variant of the SV40 large tumor antigen, facilitating proliferative growth at 33 °C and differentiation at 37 °C. These immortalized cells reveal various characteristics of differentiated glomerular endothelial cells when cultured at 37 °C and contain nondiaphragmed fenestrae, which is a unique feature of glomerular endothelial cells [Rops et al., 2004]. The creation of this cell line offers great potential for the development of a renal assist device as developed by Humes et al. Also the use of xenogeneic cells offers potential for this purpose, if the ban on research using xenogeneic cell/tissue sources would be lifted.
The Department of Nephrology of the Leiden University Medical Center is involved in a European project for the breeding of genetically engineered immune compatible pigs. The use of these xenogeneic cells might be feasible, since Humes et al. already have demonstrated (the other way around) that human cells for RAD treatment of uremic pigs can take over the porcine kidney function [Humes et al., 2002]. Nevertheless, additional research would be required to investigate the performance of such porcine cells. Again, obviously, this approach can only be followed if the ban on research using xenogeneic cell/tissue sources would be lifted.
At the UMCG Broekema et al. [2005] examined the use of extrarenal stem cells. They examined the significance of bone marrow-derived cells (BMDC) in the repair process following acute renal failure. They hypothesize that the severity of renal damage and the postischemic recovery time are determinants of tubular BMDC engraftment. They used a model of unilateral renal Ischemia/Reperfusion (I/R) in rats reconstituted with R26-human placental alkaline phosphatase (hPAP) transgenic bone marrow, in which tubular BMDC engraftment was quantified and characterized with increasing severity of damage and in time. The results indicated that the BMDC engrafted the tubular epithelium and acquired an epithelial phenotype. Tubular epithelial BMDC engraftment increased with longer ischemic time, indicating that tubular epithelial BMDC engraftment increases with the severity of damage. The number of circulating progenitor cells doubled early after I/R injury and was followed by a transient increase in tubular epithelial BMDC engraftment. The latter positively correlated with morphological recovery of the kidney over time. These findings could contribute to the development of a cell therapy that is focussed on an enhanced natural repair of the kidney.
At the University Medical Centre Utrecht (UMCU), the research group of Prof. Verhaar examined whether bone-marrow (BM)-derived cells contribute to glomerular repair. A rat allogenic BM transplant model was used to allow tracing of BM-derived cells using a donor major histocompatibility complex class-I specific mAb. In glomeruli of chimeric rats they identified a small number of donor-BM-derived endothelial and mesangial cells, which increased in a time-dependent manner. Induction of anti-Thy-1.1-glomerulonephritis (transient mesangial and secondary glomerular endothelial injury) caused a significant, more than fourfold increase in the number of BM-derived glomerular endothelial cells at day 7 after anti-Thy-1.1 injection compared to chimeric rats without glomerular injury. The level of BM-derived endothelial cells remained high at day 28. They also observed a more than sevenfold increase at day 28 in the number of BM-derived mesangial cells (specialized cells around the blood vessels in the kidneys fulfilling several functions). BM-derived endothelial and mesangial cells were fully integrated in the glomerular structure. These data indicate that BM-derived cells participate in glomerular endothelial and mesangial cell turnover and contribute to microvascular repair. These findings provide novel insights into the pathogenesis of renal disease and suggest a potential role for stem cell therapy [Rookmaaker et al., 2003; Rookmaaker et al., 2004].

Furthermore, the group of Verhaar has just obtained a grant (250.000 euro) to perform a new project (financed by the DKF) that further explores the role of BM stem/progenitor cells in chronic kidney disease (CKD). They thus focus on an approach that enhances the regenerative capacity of the kidney (renal endothelial regeneration) by administering autologous BM-derived stem/progenitor cells to the patient. The objectives of the project are:

1) To investigate whether administration of stem cells from healthy donors prevents CKD progression in a rat CKD model.
2) To investigate whether in CKD animals impairments in stem cell function or altered stem cell differentiation (stem cell dysfunction) hampers the therapeutic efficacy or even induces adverse effects.
3) To explore whether stem cell dysfunction can be modulated in vitro, using substances known to improve endothelial progenitor cell function in CKD patients, such as statins, RAAS inhibitors, and substances that increase NO availability or reduce oxidative stress.
4) To explore whether in vivo modulation of the systemic environment and/or the stem cell compartment enhances the endogenous regenerative capacity of the kidney.

Other research related to glomerular and tubular repair concerns the project of Dr. E. Popa (Dept. of Pathology and Laboratory Medicine, UMCG) which aims to identify renal endogenous stem cells (RESC) and the mechanism of maturation into renal tissues. Whereas Dr. Popa looks at endogenous stem cells in tubular repair, other investigators focus on alternative mechanism of repair, such as hematopoietic stem cells (Dr. S. Florquin, Dept. of Pathology, AMC), vascular progenitor cells (Dr. M.C. Verhaar, Dr. J.A. Joles, Depts. Of Vascular Medicine and Nephrology & Hypertension, UMCU) or at factors that are involved in transdifferentiation of resident renal cells (Dr. R. Goldschmeding, Dept. of Pathology, UMCU). Although all these studies hopefully will increase our understanding of the local environment that favors stem cell proliferation and differentiation, they do not specifically focus on tissue maturation for the application of tissue engineering [Boomker and Van Luyn, 2006]. According to Dr. Van Luyn, many answers still have to be answered in order to be able to develop an artificial kidney: ‘Can the renal cells properly attach to the material that forms the tubular structure? How long do the cells remain alive? What is the reaction of the cells being in contact with streaming fluid? Can the cells produce and excrete relevant proteins? Is it feasible to use autologous renal cells or bone marrow stem cells?’ [www.umcg.nl]. Boomker has listed the main challenges to be addressed in the coming 10 years in order to develop an artificial kidney:

a) Development ‘non-fouling’ membranes, i.e. membranes that do not activate blood coagulation and are resistant to protein adhesion.
b) Establishment of attachment and long term function of renal epithelial cells or endothelial cells to novel membranes.
c) The isolation and cultivation of (renal) precursor cells or stem cells, as well as strategies to differentiate adult or embryonic stem cells into renal tissues on scaffolds.
d) The development of smart scaffolds that can provide instructive signals to cells, such as proliferation-inducing signals, differentiation factors or cell shape changes on demand.
e) Enhanced (autologous) vascularization of the bioartificial kidney, as this is of vital importance for long-term survival of the construct and will probably also contribute to immunological tolerance for the (partly) allogeneic bioartificial kidney implant.

5.3 Conclusion

The kidney has multiple functions. Next to the excretion of waste substances, it also provides the overall important homeostasis of the blood through a sophisticated system of hormone excretion and re-absorption of minerals, water and proteins. Current hemodialysis therapy, which is the standard treatment for patients with end stage renal disease, does not provide the latter and, as a consequence, is associated with considerable morbidity and mortality. Two systems are under development that are expected to improve the renal replacement therapy and may lead to higher survival rates in patients that are waiting for a kidney transplantation. One system uses a pure medical device based approach. It mimics the excretion and reabsorbtion function of the kidney by means of double filtration membranes. One membrane functions like a 'classic' hemofiltration unit. The second membrane is designed to reabsorb substances from the hemofiltrate, which are lost in the 'classic' hemodialysis. The selectivity of the membranes can be vastly improved by new production techniques. Smart nano-membranes can be designed to selectively pass molecules, not only based on the size of the molecules, but also on dielectric properties of the molecule.
A second possibility is formed by the use of living cells or tissues. The cell based approaches that are currently in development can be divided into the following categories: I) Repair of the kidney by infusion of stem cells, II) Transplantation of fetal kidney tissue, III) Use of extracorporeal cell-coated devices, IV) Use of in vivo renal cell-coated matrixes. The most promising approach for the near future is likely to be the use of extracorporeal cell-coated devices, since this is the only approach that has entered clinical trials worldwide. This principle is based on a tissue-engineered bioartificial bioreactor that consists of a confluent layer of cultured proximal tubule cells seeded on the luminal side of multiple polysulfone hollow-fibers. This bioreactor is combined with a conventional hemofilter and acts to mimic the process of tubular reabsorption. The results obtained in the clinical trials (USA) indicate that this approach is effective. In the Netherlands a similar approach is aimed to be developed as part of the BioMedical Materials Program. Although the ultimate goal is to develop an implantable bioartificial kidney, the first big milestone will be the creation of such an extracorporeal artificial bioreactor. Nevertheless, this program has just started and clinical studies in the Netherlands with cell-based artificial kidneys to repair, replace or reconstruct renal function are not expected in the coming 5-10 years.

5.4 References

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6. Pancreas

The pancreas is an elongated organ in the abdomen with a function in the digestion of food and in the regulation of blood glucose levels. Bicarbonate ions are secreted to neutralize the acidic fluid coming from the stomach. Digestive enzymes are drained directly into the duodemum and convert proteins to amino acids and small peptides (trypsinogen), polysaccharides to mono- and disaccharides and oligosaccharides (amylase), fat to monoglycerides and fatty acids (lipase). Pancreatic failure thus affects predominantly the absorption of large molecules.
The β-cells of the pancreatic Islets of Langerhans secrete insulin, which increases entry of glucose into cells, glycolysis and storage of glucose, and so reduces blood glucose levels. It inhibits breakdown of fat and speeds up its formation and it has an anabolic action on protein metabolism. Also a protein with an unknown function, amylin, is produced by the β-cells.
The α-cells of the pancreas secrete glucagon. Glucagon raises blood glucose levels by actions opposite to those of insulin. Paradoxically glucagon stimulates insulin secretion by the pancreas, this action tending to lower blood glucose levels.
The pancreatic δ-cells form one of the locations that produce somatostatine, a peptide hormone that regulates the endocrine system and affects neurotransmission and cell proliferation.
Dysfunction of the β-cells is the cause of diabetes, type 1 and 2. It is estimated that currently some 194 million people worldwide have diabetes and that this will increase to 333 million by 2025 (http://www.eatlas.idf.org/webdata/docs/Atlas%202003-Summary.pdf). So far, development of an 'artificial pancreas' is focusing on substitution of the insulin producing function only.

6.1 Medical device-based approach for function recovery

Closed loop systems
Diabetes patients using insulin have to take fingertip blood samples several times a day, followed by the determination of the blood glucose level and the subcutaneous injection of insulin. The goal is to keep the blood glucose concentration within the physiological range (6-7 mmol/l), thus preventing the long term problems associated with hyperglycemia and the short term risks of hypoglycemia. For over 40 years now studies have been performed on the development of a closed-loop glucose measurement and insulin delivery system, 'an artificial pancreas'. An ambulatory automated insulin delivery system is a dream for many diabetes patients from the viewpoint of being released from daily blood pricking and insulin injecting. From the medical point of view benefit can be found in the fact that tight glycemic control reduces and delays serious secondary complications [Hovorka, 2006; Steil et al., 2004a; Tanna et. al., 2006]. Also the short time risk of hypoglycemia could be decreased by a continuous controlling system.
Closed-loop feedback systems are systems that use mathematical algorithms to convert measurement results into outputs like administering medication. In medical settings these systems lead to circumvention of the need for patient action/compliance and/or professional interference. In recent years substantial progress has been made in the development of insulin pumps, algorithms, and sensors bringing closed-loop systems for the assistance or replacement of pancreas functions nearer to the market.

A closed-loop system for insulin administration consists of a continuous glucose monitoring device based on a glucose sensor, an algorithm-based dose controller and an insulin pump for automated insulin delivery. The information in this report on the closed-loop system for insulin administration was adapted and updated from our previous report on new and emerging medical technologies [Geertsma et al., 2007].

6.1.1 State of development

Glucose sensors

Since 1999 continuous (or frequent intermittent) glucose monitoring systems enabling retrospective data analysis of blood glucose profiles are commercially available for short time diagnostic use and treatment optimization. Most systems can also be used as an alarm for blood glucose levels exceeding the physiological range.

These systems are minimally or non-invasive and measure glucose concentration in the interstitial fluid of subcutaneous tissue. Main approaches for sampling are:

- subcutaneous insertion of an electrochemical sensor,
- subcutaneous insertion of a microdialysis catheter which is perfused with dialysate in which the glucose concentration is measured electrochemically outside the body,
- transdermal extraction of interstitial fluid in which the glucose concentration is measured electrochemically.

Measurements are mostly based on the generation of hydrogen peroxide from glucose via the enzyme glucose oxidase, which is specific for glucose. The electric current generated is measured. Regular calibration using finger sticks and common glucose meters is however still necessary. Most subcutaneous sensors are disposable and last for three to four days. The sensor is connected to a non disposable monitor. Data on glucose levels, insulin dosing, errors and alarms are stored and can be downloaded afterwards.

For glucose monitors precision, accuracy, sensitivity and stability are important, as well as calibration requirements, availability of results, longevity and robustness [Chia and Saudek, 2004; Hovorka, 2006]. Clark error grid analysis is a technique often used to compare the accuracy of sensor readings with the accuracy of standard glucometer readings [Chee et al., 2003; Hovorka, 2006]. Continuous measurement of the glucose level is not compulsory, measuring every 10-15 minutes seems to be sufficient [Hovorka, 2006]. Glucose monitoring systems on the market are listed in Textbox 6.1. Other products are under development, including minimal and non-invasive devices, see Textbox 6.2. However, until now the results of developing really minimal or non-invasive glucose monitors have been rather disappointing.

Several other sensors are being developed that can be implanted for a longer or shorter term. Medtronic Minimed, Synthetic Blood International and Dexcom have done research in this field. The DexCom™ LTS system is implanted under the skin in the abdomen and is designed to function for up to one year, after which it must be replaced by a physician. Readings are transmitted wirelessly to a hand-held receiver. The system is currently under clinical investigation [Chia and Saudek, 2004; Diabetesnet, 2006a].

Other devices based on various technologies have not yet reached the market: the use of ultrasound (to increase permeability and transdermal transport), fluorescence, near or middle infrared light (to measure glucose on the base of absorption, reflection or optical rotation), or ‘smart’ glucose sensitive gels (that show reversible viscosity change under influence of glucose leading to controlled release of insulin [Diabetesnet, 2006a; Tanna et al., 2006]).

For the European market, blood glucose monitors must meet the European standard EN-ISO-15197: 2003 In vitro diagnostic test systems – Requirements for blood-glucose monitoring systems for self-testing in managing diabetes mellitus.

Textbox 6.1: Glucose monitoring systems on the market *[Clark, 2006; Diabetesnet, 2006a]*

- CGMS (Continuous Glucose Measurement System) Gold™ of Medtronic Minimed, Inc.: mainly for short time use, in combination with standard glucometer readings. A subcutaneous sensor for 24-72 hour use sends every ten seconds an electric signal to the monitor. The monitor averages the measured values every five minutes and stores these mean values together with information added by the patient. Data are afterwards downloadable by medical professionals for optimizing therapy.
- Guardian® RT Continuous Glucose Measurement System of Medtronic Minimed, Inc.: patient owned, for long-term use. A radio frequency transmitter sends real-time glucose values from the sensor to the monitor every five minutes. The system includes alarms. Eight hours of glucose values can be viewed by the patient on the screen of a computer for evaluation of the effects of food, exercise and lifestyle. A minimum of two finger stick measurements are necessary to calibrate the system every 24 hours. Finger sticks are also required prior to insulin delivery decisions. The disposable sensor can be used up to three days. After controlled market release in USA and FDA approval in August 2005 distribution was expanded. A recent modification of this system is the Guardian REAL-Time monitor (FDA approval April 2006, CE), that will communicate with Minimed Paradigm 522/722 pumps.
- STS™ of DexCom Inc. is a device with a subcutaneous wire like sensor that wirelessly transmits real-time glucose readings to a hand-held receiver. The sensor must be replaced every three days. It gives high and low glucose concentration alerts and low glucose alarm. DexCom's STS™ was FDA approved on March 27, 2006. A seven day version of this system, the STS-7 System, was FDA approved on June 4, 2007. It is meant to detect trends and patterns that would not be captured with fingerstick measurements alone. However diabetics must still rely on fingerstick test to decide about insulin dosing.
- GlucoDay®S of Menarini Diagnostics: a micro pump and a biosensor coupled with a micro dialysis system and worn in a portable pouch around the patient's waist. It needs one point calibration per 48 hour monitoring, two points are recommended for real-time monitoring. Results can be shown on the display and/or transmitted to a computer. The instrument has an alarm function (buzzer or vibration) for hypo- and hyperglycemia. The GlucoDay® is meant for clinical/ICU or diagnostic investigations and for analysis of measurement results by a health care team and optimizing the insulin regimen. (CE-marked, Class IIA, available in the Netherlands and several other European countries, not in the USA).
- GlucoWatch® G2® Biographer of Johnson & Johnson/Animas: a noninvasive 'wristwatch' sensor collecting glucose from interstitial fluid by reversed iontophoresis, received FDA approval in 2001. In gel collection discs glucose reacts catalyzed by glucose oxidase. The lifetime of the discs is up to 13 hours. Glucose readings are provided every ten minutes but values of two 10-minutes periods are averaged. It includes an alarm function (sound). The sensor is indicated for detection and assessment of hypoglycemia and hyperglycemia episodes, facilitating therapy adjustments. It received FDA approval but is not meant to replace a regular blood glucose meter. It is available in the USA and in the UK

Textbox 6.2: Glucose monitoring systems under development

- The FreeStyleNavigator™ of Abbott Diabetes Care/Therasense is currently under review for FDA approval. It consists of a sensor placed just under the skin for several days with a plastic sensor mount adhered to the skin like a patch. The second part is a wireless (RF) transmitter and the third part a small receiver designed to display glucose values and trends, with data storage and alarm function.
- SCGM1 of Roche Diagnostics: subcutaneous continuous glucose monitoring for up to 120 hours, based on the micro dialysis technique. The system is worn using a belt pack.
- SpectRX, Inc. is developing the Altea MicroPor™ Laser which creates microscopic pores for the interstitial fluid to cross the outer skin barrier. Glucose can subsequently be measured in an external patch containing a glucose sensor. The SpectRx personal glucose monitoring technology has been licensed to Abbott Laboratories. At the moment this monitor has not yet passed the stage of clinical investigations [Diabetesnet, 2006a].
- Sensors for Medicine and Science, Inc.® (SMSI®), is developing a watch like glucose sensor to be implanted under the skin in an outpatient procedure. The sensor measures interstitial glucose every few minutes and communicates wirelessly with a small external reader, allowing the user to monitor glucose levels continuously or on demand. The reader is designed to be able to track the rate of change of glucose levels and warn the user of impending hypo- or hyperglycemia. The target operational life of the sensor implant is 6-12 months, after which it must be replaced. Pre-clinical studies for FDA Pre-Market Approval (PMA) are underway.
- Glucon Inc. developed Aprise™, a continuous, non-invasive, glucose monitoring device intended for self monitoring diabetic patients. It provides a glucose measurement every three seconds and uses photoacoustic (optical and sound-based) technology that enables blood glucose measurements to be accessed directly from inside a peripheral blood vessel. Aprise is in clinical trials, but it is not clear how developments on this product proceed.
- Sontra was developing a continuous non-invasive glucose monitor, the Symphony™ Diabetes Management system, together with Bayer Diagnostics. The system was based on diffusion of glucose through ultrasonically permeated skin and analysis in a biosensor patch with a radiofrequency transmitter and a glucose meter. Sontra expected to start FDA clinical studies in 2006 in ICU patients, but in December 2006 they went out of business.
- GluMetrics is developing glucose sensors based on fluorescence: the GluGlow™ technique. GluGlow is a boronic acid-based polymeric material, which glows in the presence of glucose. Their first device, GluCath™, is intended to be used for continuously monitoring of hospitalized patients in the ICU. It is a catheter device that uses a thin fiber-optic cable incorporating GluGlow. A next development, BetaGlow™ is meant to comprise the sensing component of a closed-loop system. All Glumetrics products are still under development.

Algorithm-based dose controllers

An automated feedback system is necessary that translates blood sugar level (BSL) readings into appropriate insulin dosage. The system must be based on a validated algorithm. This algorithm should mimic the response of the pancreatic ß-cell on glucose levels, which includes e.g. 'first phase insulin release', 'second phase insulin release', the so called 'glucose priming' effect, and ß-cell inhibition in proportion to plasma insulin level [Steil et al., 2004a]. There are two main categories of control algorithms used for insulin closed loop systems: 'proportional-integral-derivative' (PID) controllers and 'model predictive control' (MPC) [Bequette, 2005; Schaller et al., 2006; Steil et al., 2004a].

Mimicking the ß-cell response shows some complications:

- it is complicated to imitate the insulin-secretion profile for meal (BSL increasing) and exercise (BSL decreasing);
- depending on the type of sensor and its location, different delays and noise in the transmitted signals will be present;
- glucose monitoring is accompanied by a delay due to glucose diffusion and measurement (sensing delay). Thus, glucose measurements are not completely 'real-time' [Hovorka, 2006; Steil et al. 2004a; Wolpert, 2003]. This is a problem in case of large disturbances such as following daily meals. Similarly, depending on the type of pump and its location (subcutaneous or intraperitoneal), the insulin dynamics will be different [Steil et al., 2004a]. They give a delay in the peak of the glucose lowering effect due to the time necessary for absorption of insulin from the subcutaneous or intraperitoneal environment (insulin delay) and for insulin action.

The delays must be dealt with by algorithms. Noise can be reduced by the use of filters in the algorithm [Clark, 2006; Steil et al., 2004a].

There are some other factors to be dealt with using algorithms for closed-loop systems:

- the insulin sensitivity of an individual may vary substantially, e.g. due to changes in fitness or health, time of day or mental stress levels;
- insulin absorption characteristics and sensor dynamics can vary due to a new placement of the delivery catheter or sensor [Bequette, 2005];
- the performance of algorithms can be affected by factors like dietary fat (delays gastric emptying and induces postprandial insulin resistance), alcohol (suppresses hepatic glucose production), and caffeine (induces insulin resistance) [Wolpert, 2003].

An interface allowing input of information about these factors into the pump controller by the patient could add to the precision of the system, but makes the system less 'closed' [Wolpert, 2003]. Another point is that also information about the functioning (or non-functioning) of the insulin pump should reach the controller [Bequette, 2005]. Research on the further refinement of control algorithms for an artificial pancreas is still continuing [Ibbini and Masadeh, 2005].

Smart insulin pumps

External pumps can be used for Continuous Subcutaneous Insulin Infusion (CSII). The first commercially available insulin pumps for subcutaneous administration and ambulatory use appeared on the market around 1980 [Steil et al., 2004a]. Short acting insulin analogs are administered at a low rate. The insulin reservoir has to be filled by the patient every two or three days. Currently, pumps with memory allow for data downloading. An external pump delivering a rapid analog of insulin is considered 'the gold standard of insulin delivery'. Some of the newer external insulin pumps on the market are listed in Textbox 6.3. An example of a combination of a continuous glucose monitoring system communicating with an insulin pump is shown in Figure 6.1.

Textbox 6.3: External insulin pumps on the market *[Clark, 2006; Diabetesnet, 2006b]*

- 512 and 712 (larger insulin reservoir) of Medtronic Minimed, Inc.: the Paradigm Link® Blood Glucose monitor automatically transfers blood glucose results (obtained with test strips) to this pump through RF transmission. The pump's calculator recommends an insulin dose and, subsequently, the patient can simply push a button to administer the recommended dose or can select to change the dose. The patient has to administer boluses before meals and infusion rate can be manually adjusted before physical exertion etc. A Bolus Wizard® calculator gives insulin dosing recommendations. The Paradigm® 515 and 715 are updated versions of 512 and 712. The Paradigm® 522 and 722 pumps have been developed for communication with the Guardian-REAL Time continuous glucose monitoring system, as an integrated monitor/pump-device: the Paradigm REAL Time device. Patients have access to real-time glucose data directly from a display on the device and recommended doses are calculated. The pumps are FDA approved and CE-marked.
- Deltec Cozmo® insulin pump of Smiths Medical MD: can be combined with the CoZmonitor blood glucose monitor (powered by FreeStyle®-test strip technology from Abbott Diabetes Care), which can be attached to the back of the Deltec Cozmo® insulin pump to create an all-in-one device. The pump keypad and screen are used for all glucose testing functions and results. The meter reads blood sugars directly into the pump via an infrared port, and can be used during plane flights. Bolus recommendations are given after entering carbohydrates in the meal and/or blood sugar levels. The 'all-in-one' system is available in the USA only.
- OmniPod™ Insulin Management System of Insulet Corporation: a two-part system of a FreeStyle® blood glucose monitor and a device for continuous subcutaneous insulin delivery with automated canula insertion. It is worn on the skin like an infusion set and delivers insulin according to pre-programmed instructions transmitted wirelessly from the Personal Diabetes Manager (PDM), a hand-held device in which the glucose monitor is integrated. The OmniPod monitors the operation and checks blood glucose levels using FreeStyle blood glucose test strips. It includes a suggested bolus calculator. The system received FDA clearance in January 2005.
- Animas IR-1250 of Animas (FDA approved, but not yet available in Europe): the glucose meter interface ezManager Plus™ enables downloading of data from pump and glucose meter to a computer. There is not yet a direct input from blood glucose meter to pump. Animas is expecting to utilize a direct infrared (IR) port for blood glucose data transfer. Animas is developing an external micropump for subcutaneous injection of insulin which can be directly taped on the skin and is operated and programmed through a Remote Control Device.
- Accu-Chek Spirit of Roche (formerly Disetronic): to be used with Accu-Chek® Pocket Compass software with bolus calculator on a palmOne™ device and Accu-Chek® blood glucose meter. Received FDA clearance in 2005 and is now on the European market.

Figure 6.1. A combination of a continuous glucose monitoring system communicating with an insulin pump. *(source Medtronic Minimed, Inc. http://www.minimed.com/professionals/realtime/index.html)*

The intraperitoneal insulin administration by implantable pumps gives a more physiological insulin delivery. Implanting pumps is, however, more expensive and experience with implantable pumps is limited. In 1999 approximately 1000 pumps were implanted [Hovorka,

2006]. Pump pocket infections, catheter blockage and device failure may necessitate surgical removal. Stability of insulin is a point of attention.
There is only one implantable insulin pump approved and commercialized in the EU (Minimed Medtronic 2007, CE mark). This pump is implanted in the lower left quadrant of the abdomen and a 20-30 cm long catheter is placed such that the tip is in the intraperitoneal cavity. U400-regular insulin is filled across the skin into a pump reservoir every two to three months. Insulin delivery is modulated by the patient using an external programmer using RF telemetry, according to the results of self blood glucose monitoring. Bolus doses before meals can be given by pushing a button. The programmer must be synchronized with only one pump, assuring other implantable devices are not affected. The life span of the pump battery is dependent of the daily insulin delivery, but is suggested to be at least seven years.
Implantable pumps offer advantages for patients who have difficulty in maintaining consistent glycemic control, even using CSII [Catargi, 2004].
Insulin pumps must meet the European standard EN 60601-2-24:1998 Medical equipment – Part 2-24: Particular requirements for the safety of infusion pumps and controllers.

Closed-loop systems for insulin administration
Based on the abovementioned components prototypes of closed-loop systems have been developed. The two main approaches are [Hovorka, 2006]:

- Extracorporeal: subcutaneous glucose monitoring and subcutaneous insulin administration (s.c.-s.c.). This system is a minimal invasive solution that can benefit from the experience of more than 200,000 external insulin pump users. Therefore it seems to be the best possibility for widespread application.
- Implantable: intravenous glucose monitoring and intraperitoneal insulin delivery (i.v.-i.p.). Intravenous sensors implanted in the circulatory system, e.g. vena cava, are mainly for short time use in a hospital environment. There is less experience with implantable pumps delivering insulin intraperitoneally [Catargi, 2004; Hovorka, 2006; Pickup and Keen, 2002].

Due to longer delays particularly users of s.c.-s.c. systems will have to enter information on meals or physical exertion, and due to this the loop system is not fully closed anymore. There are different ways to handle mealtime insulin delivery:

- ‘fully closed-loop’: insulin is administered by evaluating the rise in postprandial glucose.
- ‘semi-closed loop’ or ‘closed-loop with meal announcement’: patient gives information about time and size of the meal in advance and the controller advises on an insulin bolus.
- ‘closed-loop with qualitative meal announcement’: patient gives information about time of the meal and the controller switches to a more aggressive mode of insulin delivery.

Meal announcement or “feedforward control” improved results [Bequette, 2005; Hovorka, 2006; Wolpert, 2003].
A remaining restraint on the performance of an artificial pancreas is that it uses only insulin to control blood glucose levels, while the physiological pancreas uses both insulin and glucagon. It is difficult to prevent hypoglycemia without glucagon or glucose as an additional manipulated input [Bequette, 2005].

Studies with continuous glucose monitoring systems in closed-loop situations were limited in number of subjects as well as in duration [Hovorka, 2006].
In 2004 an evaluation was published of an external physiological insulin delivery (ePID) algorithm, an adoption of a PID-controller [Steil et al., 2004b]. A subcutaneous glucose sensor of MedtronicMiniMed was used, calibrated before the start of closed-loop control, and checked regularly. Six subjects received each four meals in 27.5 hours, without meal

announcement. Preprandial and postprandial (2hr) glucose levels were 5.8 ± 1.2 and 9.8 ± 1.6 mmol/l (mean ± SD), respectively. Morning glucose level after overnight control was 6.8 ± 1.0 mmol/l.
Another study published in 2004 used the sensor SCGM1 of Roche Diagnostics in a s.c.-s.c. closed-loop system with meal announcement [Galley et al., 2004]. An empirical algorithm was used that was converted to a model predictive (MPC) framework. The closed-loop system was evaluated in twelve patients treated with CSII. Control with the algorithm was compared to standard self-directed therapy over study periods of 32 hours. Each period included ingestion of four meals and quantitative meal announcement was given. The algorithm achieved a mean monitored glucose concentration of 6.9 mmol/l vs. 6.2 for self-directed therapy. It reduced the number of hypoglycemia interventions. During algorithm therapy 56% of GCMS1 values were within the 5.0-8.3 mmol/l range compared with 33% with the self-directed therapy.
In five critically ill patients on an intensive care unit a study was performed in 2003 using GCMS as a sensor, coupled with a proportional integral control algorithm based on a sliding scale approach for automatic intravenous infusion of insulin [Chee et al., 2003]. In four patients manual intervention was needed due to the real-time sensor reading of blood sugar levels deviating more than 20% from the glucometer value. The conclusion of this study was that more work was needed for the refinement of the algorithm and the improvement of real-time sensor accuracy, and that the algorithm was not yet suitable for use in ambulatory patients.
In 2004 a study was published on the testing of an i.v.-i.p. closed-loop system in four diabetes patients over 48 hours. A long-term sensor system (LTSS) was used, containing a sensor of MedtronicMiniMed, and was combined with an implantable physiological insulin delivery system (iPID). Quantitative information on breakfast, lunch and dinner were given. During the first 24 hours empirical tuning of the algorithm took place. During the second 24 hours control period 4% and 7% of the time was spent below 4.4 mmol/l in the postprandial period and outside meal conditions, respectively, 12% and 32% was spent in the region 4.4-6.7 mmol/l, 63% and 60% was spent in the region 6.7-13.3 mmol/l, and 20% and 2% was spent above 13.3 mmol/l [Renard et al., 2004].
Although small-scale laboratory studies with closed-loop systems have been performed, performance in routine in the clinic as well as in the home setting has yet to be demonstrated [Hovorka, 2006].

Marketing in the coming years
A personal wearable treatment system is the ultimate goal. Although there are several sensors on the market or at near approval, reliability and accuracy of the presently available monitors is not yet considered sufficient to replace invasive measurements, obtained with blood glucose meters and test strips, for decisions on insulin dosage. Currently, these sensors are promoted to detect trends and track patterns in glucose levels and for an alarm function. Sensor lifetime is still limited. For long-term use time-related effects such as increasing lag because of progression of biologic fouling or foreign body fibrosis may cause problems on the sensor surface [Ward et al., 2005]. Besides, implantable intravenous glucose sensing is invasive and may cause biocompatibility problems.
Although miniaturized external pumps as well as an implantable insulin pump are available and reliable nowadays, glucose sensors and algorithms have not yet proved to be reliable enough at the moment for real closed-loop administration of insulin [Catargi, 2004]. Nevertheless, several all-in-one devices have been introduced recently to the market or are pending for FDA clearance. They consist of a continuous glucose monitor, a dose advising controller and a smart insulin pump. Finger prick glucose monitoring is still necessary for

regular calibration and required for confirmation of glucose levels before insulin doses are programmed. Additionally, the patient has to enter information on meals, exertion etc. Therefore these systems have a semi-closed-loop character, advising patients on dosing.

Late 2005 the Juvenile Diabetes Research Foundation (JDRF) in the USA has launched the Artificial Pancreas Project, funding research with the aim to speed and optimize the development of a closed-loop system (www.jdrf.org). From November 2006 children in the UK with type I diabetes are being recruited to test - from January 2007- a computerised glucose sensor, to perfect the computer algorithm for use in an artificial pancreas (http://www.admin.cam.ac.uk/news/press/dpp/2006110102 ,www.bbc.co.uk / 6-11-06). The researchers hope that after a series of clinical trials over the next two years the device can be used in children in their own homes, and expect that it can be available commercially in four to seven years. This is one example of the research efforts in this field that will probably result in continuing innovations in the coming years, coming close to closed-loop systems.

Closed-loop systems for insulin administration may be used first in controlled environments like intensive care units. Systems for short-time use could include an intravascular real-time glucose sensor. For critically ill patients in the surgical intensive care unit and for diabetes patients with acute myocardial infarction tight glucose control significantly decreases mortality [Hovorka, 2006; Wolpert, 2003].
The first generation of closed-loop systems will probably not achieve complete normalization of the glucose profile. When the system can maintain or improve the level of glycosylated hemoglobin (HbA1c, an indicator of how well the blood glucose has been controlled over the past three months) and reduce the frequency of hypoglycemia it could be suited for the treatment of a small group of well motivated patients. For home treatment the device must be easy to program and to use, the user interface needs to be straightforward, the calibration of the glucose sensor needs to be easy, fault detection must be incorporated, and warnings must be provided [Bequette, 2005]. In addition, patient education will be very important to expand the patient population having the necessary advanced skills [Wolpert, 2003].

Of all diabetes patients some 40% need insulin injections [WHO, 2002]. Only for a part of these diabetes patients a closed-loop system will be the optimal therapy. On the other hand much research is performed on less encumbering ways of administering insulin, like oral, buccal (Oralin™), transdermal or inhaled (Exubera®, Technosphere®) insulin). Resulting products and advanced transplantation techniques may eventually reduce the need for parenteral insulin administration. Furthermore it is likely that the progress of development and marketing of (semi-)closed-loop systems will be influenced by reimbursement policies of third parties like healthcare insurance companies, and by user and healthcare provider acceptance.

Possible risks
The possible risks of (semi-) closed-loop systems can be clustered around several aspects of these systems.
Sensor reliability: Inaccurate glucose values or inappropriate alarms could result in inappropriate administration of insulin. Tissue reactions to implanted parts of a sensor can interfere with accuracy and reliability [Wolpert, 2003]. Therefore lifetime is limited and regular calibration of non- or minimal invasive monitors is still required. Until now FDA approved the use of continuous glucose monitors only as a supplement to daily finger sticks, not as a replacement. Minimum requirements for properties as reliability and accuracy of sensors have to be determined.

Invasiveness: Implantation of sensors or pumps requires surgery and brings the risk of infections. Intravenous monitoring may cause thrombosis or embolization [Ward et al., 2005]. Implanted pumps require surgical removal after their functional lifetime. Infections, catheter blockage and device failure may necessitate early removal. Regularly a sterile refill procedure has to be performed and the stability of insulin at body temperature is a point of attention. The experience with implantable pumps is limited.
Algorithms: Blood glucose is complicated by several variables like food intake, physical activity, stress, illness and sleep. Rapid changes in blood glucose are difficult to be dealt with by algorithms without patient input of information. Failure of algorithms may cause substantial risk because people may put a lot of faith in advised or recommended doses. Good control by an algorithm may bring along the risk of masking technical operating problems [Bequette, 2005]. Information about the functioning of the pump should reach the controller.
Wireless information transfer: Radio frequency (RF) transmission of data may give interference with cell phones or other radio traffic and can give problems inside planes. Interference with other medical devices and with devices of other patients must be made impossible. European standards in this field have been developed by ETSI - European Telecommunications Standards Institute.
User interface: The user interface must be straightforward and programming and calibration must be easy. The patient must be capable to deal with the information provided by the system and must be well trained. In addition, it should not be possible to push buttons, such as for bolus injections, inadvertently. Small pumps with small buttons and displays may give ergonomic problems, especially for elderly people or people with a reduced vision.
Data storage: Battery removal or static electricity may cause loss of stored settings and historical data.

At the moment there are no really-closed-loop systems for diabetics that are ready for marketing. Although the newly developed systems are not yet perfect, these near-closed-loop systems may result in better blood glucose levels than can be reached with five times daily finger pricking and insulin injecting. For each patient a risk-benefit analysis will be important before deciding for a (semi-) closed-loop system. Closing the loop would entirely overcome the necessity of patients to occupy themselves daily with glucose monitoring, insulin dosing and understanding complex information, but it must be very sure that such a system brings no risks of catastrophic failure [Clark, 2006]. Systems not measuring brain glucose and not using glucagon as a second hormone will probably never be ‘perfect’ [Clark, 2006; Hovorka, 2006]. At the moment there is no widely accepted system to assess specifically the performance of closed-loop systems for insulin delivery, although recently a grading system to assess closed-loop glucose control from the clinical point of view was suggested [Chassin et al., 2005; Hovorka, 2006]. Experience with near-closed-loop systems will provide more information for deciding whether and how to close the loop.

Regulation
Closed loop systems are intrinsically characterized by a close cooperation of different devices: a sensor for measurement of diagnostic parameters(s), a controlling algorithm and a therapeutic device. Some of these systems have to deal with very complex physiological processes, which may be critical processes too (like systems for insulin therapy, or anesthesia). It is of essential importance that the system reacts promptly and properly on a diversity of physiological conditions and events. Furthermore closed loop systems taking over control means that it may take some time before medical professionals or patients detect a deviation in device functioning, or a device action that is deteriorating or fatal for the patient’s health. Therefore failing of closed loop systems may have serious consequences for

patients. This means that closed loop systems controlling critical physiological processes should have to meet very stringent requirements with regard to performance and reliability. Data transfer between the components must be impeccable. Risk assessment and clinical studies should be extensive and take into consideration all possible physiological conditions and environmental circumstances.
Because closed loop systems are complicated devices, some of which generate a considerable amount of data to be analyzed or dealt with, extensive training of medical professionals and/or patients is of essential importance.
Recently a standard on the development of closed loop systems has been published: IEC 60601-1-10 Medical electrical equipment - Part 1-10: General requirements for basic safety and essential performance - Collateral Standard: Requirements for the development of physiologic closed-loop controllers. It specifies requirements for the development (analysis, design, verification and validation) of a physiologic closed-loop controller as part of a physiologic closed-loop control system in medical electrical equipment and medical electrical systems to control a physiologic variable.

6.1.2 Applications in the Netherlands

In 2005 around 8,500 external insulin pumps were used in the Netherlands in a home setting [Hollestelle et al., 2005]. Only a few hospitals supervise patients with an implantable pump. Continuous glucose monitoring devices are used in limited numbers, e.g. in diabetic women trying to become pregnant, for children, in patients who have difficulties in controlling their blood glucose levels, or on a short-time basis for patient education. At the moment 15 patients in the Netherlands are using a Medtronic Paradigm REAL-Time system: a continuous glucose monitor in combination with a Paradigm insulin pump. The reimbursement for these integrated devices, especially the continuous-glucose-monitoring part, is difficult to be arranged: individual requests have to be submitted. Furthermore in 2007 a user evaluation study has started in 12 hospitals with the Minimed Paradigm REAL Time and with the Guardian REAL Time device of Medtronic. With these systems the patient still has to decide on the insulin dosing, based on the glucose levels displayed on the device. Therefore, this is still an ‘open-loop’ system. Other combinations of continuous glucose monitoring devices with insulin pumps are not yet used in the Netherlands at the moment [Ledegang, 2006; Medtronic, 2007a; Medtronic, 2007b; NVDO, 2006].

6.2 Cell/tissue-based approach for function recovery

6.2.1 State of development

Patients suffering from glycaemic lability despite optimized medication are the candidates for pancreas transplantation. Islet transplantation, where islet cells are harvested from brain-dead donors, processed according to a standardised protocol and infused in the recipient’s liver has also been clinically investigated, but the results are inferior to pancreas transplantation [Frank et al., 2005]. Furthermore, islet transplantation is an inefficient use of donated islet cells (for a single recipient, 2 to 4 donors are needed), so currently whole-pancreas transplantation is a better option. Because of the organ shortage, there is a stimulus for research into the possibility to use other cell or tissue sources. Basically, two approaches are being explored. The first is the use of stem cells and the second is the construction of a bio-artificial pancreas (BAP), where islet cells from different sources are contained in a device.

Human stem cells
Transplantation of islet cells can provide reestablishment of normal blood glucose levels in insulin dependent diabetic patients [Shapiro, 2006]. However, the availability of suitable numbers of donor islet cells is very low compared to the demand, therefore alternative sources of these cells have been searched for. Theoretically, human stem cells can also provide islet cells and this notion has indeed been proven in laboratory circumstances. It has been shown that a substantial fraction of an initial number of human embryonic stem cells (hES) can be guided to differentiate into pancreatic islet-like cells, that produce high amounts of insulin [D'Amour et al., 2006]. Knowledge from the signaling factors that are relevant for pancreatic development in mouse, chicken and zebrafish have been translated to a 4-step protocol that exposes the hES cells in a monolayer culture to various growth and differentiation regulating substances. The resultant population of cells produced all 5 pancreatic hormones (including insulin and glucagon), although the cells did not show a consistent response to glucose. So far, this cell source is not yet ready for clinical applications.
Other sources for stem cells that are being studied are derived from bone marrow, the lining of the pancreatic duct, liver, bile duct epithelium. Still, full and consistent control of the development of these cells into functional, insulin secreting cells has not been achieved [Noguchi, 2007].
It should be noted also, that the success of transplantation of cells depends on the removal of the cause of destruction of the ß-cells, otherwise the transplanted cells (homologous as well as heterologous) will also be destroyed. Furthermore, risks have been identified with manipulation and introduction of stem cells in patients: carcinoma development through pluripotent stem cells, DNA-changes caused by long-time culturing cycles, and development of immunological phenomena (countered by immunotolerance through chimerism).

Bio-artificial pancreas (BAP)
The bio-artificial pancreas (BAP), also referred to as encapsulated cell therapy, generally contains a number of islet cells, encapsulated by a semi-permeable membrane of which the geometry and characteristics may vary. In order to be successful it is required that the BAP has the following characteristics:
- biocompatible (e.g. no encapsulation with fibrous tissue by the body);
- good diffusional properties (e.g. for cell nutrition, to allow physiological feedback in response to glucose and to dispose of waste products);
- guarding the islet cells from allogenic or xenogenic sources against damage from host immune responses (thereby eliminating the need for immunosuppressive drugs);
- maintenance of cell viability over long periods of time;
- easy retrievability.

BAPs can be intravascular (usually as an AV-shunt) or extravascular. In the first type, blood flows through a chamber containing islet cells that are separated from the blood by a semi-permeable membrane. In the extravascular group one can discern macrocapsular devices (flat sheets, sealed hollow fibres or macrospheres) and microcapsular devices consisting of varying in numbers of islet cells (down to a single cell), suspended in a polymeric gel surrounded by a semipermeable membrane. The macrocapsular devices are usually implanted in the peritoneal cavity, or in a subcutaneous site. Microcapsular devices can be implanted in the aforementioned locations as well as in the renal capsule. Microcapsules are commonly made from alginate, a component of the extracellular matrix of brown algae. It contains the polysaccharides manuronic acid and guluronic acid, and it solidifies when droplets are exposed to solutions with calcium of barium ions. A cover of poly-L-lysine (PLL), a

polyaminoacid, surrounds the alginate and gives the capsule semi-permeable properties. PLL can be modified to change its permeability for large molecules (like immunoglobulins). However, a sharp cut-off limit is a theoretical characteristic and not easily realised in practice. A final outer layer of alginate covers unbound PLL-groups to prevent fibrotic reactions. Another approach to prevent immunological responses is by surface modification (camouflaging) of the islet cells. For instance, polyethylene glycol (PEG) can be coupled to amino groups on the cell surface and results in in-vitro protection to cytotoxic responses. [De Groot et al., 2004; De Vos et al., 2004; Silva et al., 2006; Orive et al., 2003; Narang and Mahato, 2006].
Alternatively, a transplantation space can be created prior to filling this space with islet cells [Valdez et al., 2005]. To this end a stainless steel meshed rod holding a PTFE tubular membrane is implanted subcutaneously in the anterior abdominal wall. After allowing the formation of small blood vessels around this rod for two months, the PTFE tubular membrane is removed through a small incision and islet cells are introduced into the rod.
The application of these technical and theoretical concepts in animal models (rats, mice, dogs and monkeys) and in man learns that a number of problems has to be solved. The results from animal studies show that although encapsulated islet cells performed better than non-encapsulated cells, the duration of euglycaemia was limited (from several to 6 months) [De Groot, 2004]. The intravascular devices bear the risk of coagulation and thrombus formation. A recipient needs vascular surgery and anti-coagulative therapy. Although glucose regulation can be sufficient, coagulation problems cause the intravascular devices not to be the approach of first choice. The extravascular devices do not present these problems and especially the microcapsular devices have been studied extensively. Macrocapsules suffer from an unfavourable surface – mass ratio (see hypoxia problems below).

Three aspects have been identified as being relevant to limited graft survival in microencapsulated islets [De Groot, 2004]: biocompatibility, immunoprotection and hypoxia. Frequently, the reaction of the host to the BAP results in fibrotic overgrowth of the device, which is appreciated as a lack of biocompatibility of the device. Fibrous overgrowth is a complex process of acute and chronic inflammation reactions and its intensity shows a great variation, not only between species, but also within the same species. However, this overgrowth does not show a consistent relation with device characteristics like membrane material, polymeric gel material, proces/technique used for encapsulation, capsule size, pore size, surface characteristics or coating with anti-fibrotic agents [Silva et al., 2005]. It has been suggested that, since the production of capsules has not yet achieved consistent results, cells may not always be fully covered by capsule material [Orive et al., 2003]. Also impurities in the alginate may contribute to bioincompatibility and efforts have been made to produce clinical-grade polymers [Orive et al., 2003]. Already, the American Society for Testing and Materials (ASTM) has developed some standardisarion documents on this topic.
Lack of immunoprotection is also related to graft failure. Although membranes can be engineered to block large proteins like immunoglobulins, it has been shown that islets (especially xenografts) secrete proteins that can pass the membrane and attract macrophages. These host cells in turn release toxic cytokines that again pass the membrane and may harm the graft cells. Furthermore, these macrophages can contribute to fibrous overgrowth.
Finally, hypoxia hampers optimal cell function, including insulin secretion. Encapsulated cells that are implanted subcutaneously or in the abdominal cavity depend on passive diffusion of oxygen. [Silva et al., 2005]. Therefore, the design of a microcapsule should take into account that the distance from the cell to the surface of the capsule is limited (around 200 μm).

Up to date the clinical experience with encapsulated islet transplantation is limited, yet growing. The first report (application in a single patient) came from the USA [Soon-Siong, 1994]. AmCyte has started a Phase I/II clinical trial (3 patients) in 2003 in Toronto, Canada [AmCyte, 2003]. A report of the first patient was presented in 2005 [AmCyte, 2005]. In 2005 Novocell started a phase I/II clinical trial (12 patients) in San Antonio, USA [Novocell, 2005] and the first results were reported in 2006 [Novocell, 2006]. In 2006, 2 cases were reported from a trial in Perugia, Italy. The report mentioned reduced insulin use and prevention of frequent weekly hypoglycaemic episodes [Calafiore, 2006].

Whereas the abovementioned studies used human islets, xenograft islets have also been used: Mexican 4-year experiences in 12 patients were reported in 2005 [Valdez, 2005]. The initial report of this study raised debate regarding the ethical issues of clinical trials with xenotransplants [Check, 2002]. Still, 2 patients became insulin independent for a few months and 6 patients showed a significant reduction in insulin requirement [Valdez, 2005]. In 2007 Living Cell Technologies (LCT) from New Zealand announced the start of a trial (6 patients) in Moscow, Russia with encapsulated porcine islet cells [LCT, 2007].

6.2.2 Applications in the Netherlands

To the best of our knowledge clinical applications are currently not performed in the Netherlands.

6.3 Conclusion

A (bio-)artificial pancreas would improve the quality of life of insuline dependent patients and would have medical benefits. For over 40 years now, studies have been performed on the development of a closed-loop glucose measurement and insulin delivery system. In the last decennia progress has been made in the development of essential components: glucose monitors and insulin pumps. Both are commercially available, including dose advising algorithms and data management options, and the application possibilities become more sophisticated year after year. However, fully closed loops systems are still not reliable and sufficiently accurate to be marketed. This is mainly due to problems with long term glucose measurement and to the complexity of dose controlling algorithms that have to respond to many different physiological circumstances. In the Netherlands, 15 patients are using a continuous glucose monitor in combination with an insulin pump. Furthermore, in 2007 a user evaluation study has started in 12 hospitals a similar system. With these systems the patient still has to decide on the insulin dosing, based on the glucose levels displayed on the device.
Cell-base therapeutic options include the use of stem cells and the construction of a bio-artificial pancreas (BAP). Therapies for diabetes based on stem cells have yet not reached maturity and are still in the laboratory phase. BAPs can be intravascular or extravascular. The intravascular devices bear the risk of coagulation and thrombus formation and are currently not the approach of first choice. The extravascular devices do not present these problems and especially microcapsular devices have been studied extensively. Clinical investigations with BAPs are still scarce, but have been carried out in the USA, Canada, Italy, Mexico and Russia. The latter two studies used porcine cells, which is not acceptable for ethical reasons in many countries including the Netherlands. To the best of our knowledge, clinical applications using a cell or tissue based artificial pancreas are currently not performed in the Netherlands.

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Manufacturer's websites:

Abbott	http://www.abbottdiabetescare.com (access date 12-02-07)
Animas	http://www.animascorp.com/index2.shtml (access date12-02-07)
Glucowatch	www.glucowatch.com (access date 12-02-07)
Dexcom	http://www.dexcom.com/ (access date 12-02-07)
Glucon, Inc	www.glucon.com(access date 8-2-07)
Insulet Corp.	http://www.myomnipod.com/ (access date 12-02-07)
Medtronic	http://www.minimed.com (access date 12-02-07) http://www.minimed.com/professionals/realtime/index.html (access date 22-2-07)
Menarini Diagnostics	http://www.menarini.com (access date 8-2-07)
Roche	http://www.roche-diagnostics.com/ (access date 12-02-07)
Sensors for Medicine and Science,Inc.	http://www.s4ms.com/glucose_personal.htm (access date 8-2-07)
Smiths Medical	http://www.smiths-medical.com/ (access date 12-02-07)
SpectRX	http://www.spectrx.com/cgms.asp (access date12-02-07)

Abbreviations

BSL	blood sugar level
CGMS	continuous glucose monitoring system

CSII	continuous subcutaneous insulin infusion
HbA1c	glycosylated hemoglobin, a measurement used to reflect glucose levels over 8 to12 weeks
ICU	intensive care unit
LTSS	long-term sensor system
MPC	model predictive control
PI	proportional integral
RF	radio frequency
STS	short-time sensor

7. Bladder

The urinary bladder is a hollow, muscular organ located in the pelvic floor and is part of the lower urinary tract. Other anatomical structures of the lower urinary tract are the urethra, internal urethral sphincter, external urethral sphincter, and ureters. The ureters drain the urine from each kidney into the bladder. The urethra is the outflow tract connecting the bladder to the exterior. The urethral sphincter enables the tight closure of the urethra.
The bladder itself is composed of four layers. The transitional epithelium or urothelium, which lines the interior surface of the bladder, is in contact with the urine. Under the epithelium is the lamina propria, a layer of connective tissue, smooth muscle and blood vessels. The third layer is the muscularis propria of detrusor muscle that forms the wall of the bladder. The outermost layer consists of a fibrous adventitia and a visceral peritoneum.

Bladder function: micturition and continence
The function of the bladder is urine micturition (periodic evacuation of urine) and continence (storage). The bladder can store approximately 500-600 ml of urine for 2-5 hours. Once the bladder contains 150-300 ml, the urge to micturate is developed and signalled. Although the choice when to urinate is under voluntary control, once decided to do so the external urethral sphincter is voluntary relaxed and the autonomic nervous system causes detrusor contractions in the bladder wall, resulting in micturition. When the bladder is empty, the detrusor relaxes and the sphincters contract to close the urethra.
Normal bladder function involves a unique combination and interaction of autonomic and somatic functions. It is mediated by neural circuitry located in the brain and lumbosacral spinal cord [De Groat, 1990]. The lower urinary tract is controlled by three sets of peripheral nerves: sympathetic (hypogastric), parasympathetic (pelvic), and somatic (pudendal) nerves. Together, this complex system regulates continence and micturition and protects the upper urinary tract [Yoshimura and Chancellor, 2003].

Bladder dysfunction
Actual damage of the peripheral or central nervous system, or disruption of the finely tuned balance between inhibitory and excitatory stimuli, results in continence and/or micturition disorders. Neurogenic bladder refers to a malfunctioning bladder due to neurologic dysfunction. This is often associated with spinal cord diseases, brain diseases, or peripheral nerve diseases, but may also be caused from internal or external trauma. Affected patients will demonstrate symptoms of urinary frequency-urgency, urge incontinence, and urinary retention, and complete micturition is problematic. If a lesion is located at spinal cord level Th12 or above, patients have a reflex bladder. Reflexes which partly control the bladder are still intact, so that when the bladder is above a certain level it micturates automatically. With an injury below the Th12 level, patients have an areflexive bladder and sphincter paralysis. The spinal reflexes are lost, the bladder has no muscle tone and does not contract to empty automatically. Instead, it continues to fill and micturition is usually inefficient.

7.1 Medical device-based approach for function recovery

Several medical devices and techniques have been developed to restore bladder function. Whereas mechanical medical devices are limited to treating symptoms of organ dysfunction,

devices using electrical stimulation can exert control over muscles and their neural control systems restoring function to persons with neurological or sensory impairment. The basis of electrical stimulators or neural prostheses is the application of electrical current pulses which generate artificial action potentials in axons of (peripheral) nerve fibres or neurons in the spinal cord by depolarisation of the cell's membrane. A detailed explanation of the basic principles of neuromuscular stimulation is provided in Appendix A.1. Nowadays, the use of implantable electrical medical devices for the neurophysiologic control of the bladder has reached a stage of successful clinical application.
Five primary locations can be identified where electrical stimulation is applied: on peripheral nerves, sacral roots, in the spinal cord itself, in/adjacent to the urethra, and on the skin. In general two techniques can be distinguished: neurostimulation and neuromodulation. Neurostimulation is used to control the nerve fibres for bladder micturition in spinal cord injury subjects. Neuromodulation is used to achieve immediate reflexes/responses to increase bladder continence [Fall and Lindstrom, 1991; Leng and Chancellor, 2005; Blok et al., 2006].

7.1.1 State of development

An overview will be given of the state of development concerning electrical, hydraulic, mechanical and other types of medical devices for restoring bladder function. Devices, stimulation techniques, and some surgical techniques that are likely to affect the field in the future are described.

7.1.1.1 Electrical medical devices for neurostimulation

Sacral nerve root stimulation

In the early/mid 1980s sacral nerve root stimulation was reported in patients with spinal cord injury to enable micturition [Brindley et al., 1982; Brindley et al., 1986]. The sacral root stimulation system was developed by Brindley and is currently known as the Finetech-Brindley Bladder System (Finetech Medical Ltd, Welwyn Garden City, UK) or Vocare™ Bladder System (NDI Medical, Inc., Cleveland, OH, USA). Since then some 3000 of these devices have been implanted [Donaldson et al., 2007; Rijkhoff, 2004]. In general, it has provided good results (in some cases for over 15 years) and achieved sustained clinical use in spite of a few drawbacks [Brindley et al., 1986; Brindley, 1994; Brindley, 1995; Creasey et al., 2001; Egon et al., 1998; Kutzenberger et al., 2005; Van Kerrebroeck et al., 1997; Vastenholt et al., 2003].
The implantable components of the Finetech-Brindley Bladder System include tripolar 'book' electrodes, leads, and a passive receiver/pulse generator. The implant has no batteries and the various components are encased in silicone. The external portable control unit consist of an antenna connected to an external transmitter/controller device which allows programming of the stimulation parameters by a clinician, and provides the power for nerve root stimulation. Micturition is initiated after the external control unit is activated by the user. Implantation of the electrodes is technically demanding, requiring microsurgical techniques. The implantation of electrodes is usually supplemented by severance of posterior nerve roots (rhizotomy). This has several substantial advantages, including increased bladder capacity and compliance, reduced reflex incontinence, and protection of the upper urinary tracts from ureteric reflux and hydronephrosis [Brindley, 1994]. The intervention has certain drawbacks as well, such as abolition of reflex defaecation, and in male reflex erection and reflex ejaculation. However, in many spinal cord injury subjects these reflexes are not adequately functional and can be restored by other techniques. In recent years, patients have often objected to this intervention, not only because of the above mentioned drawbacks, but also because it is irreversible. In addition, researchers in the field of spinal cord repair proclaim means of curing spinal cord

injury in the (foreseeable) future. A detailed description of the Finetech-Brindley Bladder System and associated surgical techniques is provided in Appendix A.2.

Sacral nerve neuromodulation

Sacral nerve neuromodulation is used as therapy for non-neurogenic bladder dysfunction. Techniques for accessing the sacral nerves through the sacral formina were developed making the implant procedure faster and less invasive [Schmidt et al., 1990]. Initial devices were the Itrel-I™ and Itrel-II™ pulse generators developed by Medtronic (Minneapolis, MN, USA). Currently, the InterStim® and InterStim® II (smaller in size) Implantable Neuro-stimulators are marketed (Figure 7.1). InterStim® was first introduced in the early 1990s and presently more than 25.000 implants worldwide have been performed [Oerlemans and Van Kerrebroeck, 2007]. The implantable system is comprised of a battery-powered electrical stimulator, an extension cable (not for the InterStim® II), and a lead with electrodes. Subsequent adjustments of the stimulation parameters can be accomplished easily and non-invasively with an electronic programming device. The battery can run for about five years and can be replaced during an outpatient procedure. Sacral nerve neuromodulation is only effective in a subset of patients with lower urinary tract dysfunction, therefore all patients are initially evaluated with a percutaneous electrode connected to an external stimulator to assess their response to this treatment before permanent implantation [Janknegt et al., 1997].

The number of technical problems and hence the complication rate has been significantly reduced with changes in the hardware and fine-tuning of the surgical technique. Hardware improvements concern a new battery type and the introduction of a new lead [Spinelli et al., 2003a; Spinelli et al., 2003b]. Surgical advancements, such as a minimally invasive approach to percutaneous placement of the lead under local anaesthesia have led to an increase in patient comfort and a reduction in time needed to perform the procedure [Scheepens et al., 2001; Spinelli et al., 2003a]. Medium-term and long-term outcomes are promising and demonstrate that sacral nerve neuromodulation is safe and effective [Dasgupta et al., 2004; Elhilali et al., 2005; Siegel et al., 2000; Van Voskuilen et al., 2006; Van Voskuilen et al., 2007]. A detailed description of sacral nerve neuromodulation is provided in Appendix A.3.

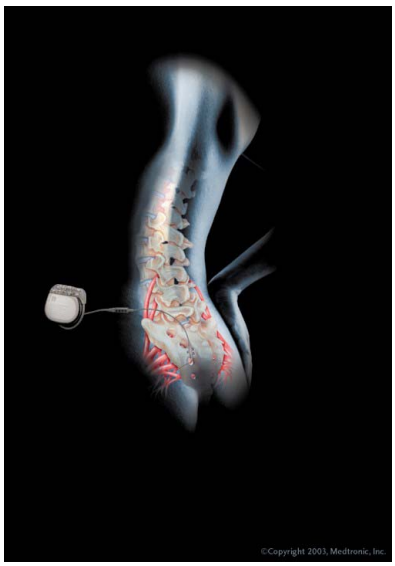

Figure 7.1 InterStim® therapy. Anatomical position of the lead and the neurostimulator (on the left). InterStim® product family (on the right) consists of two implantable models (InterStim® II and InterStim® Implantable Neurostimulator) to meet the needs of individual patients, the InterStim iCon™ Patient Programmer allowing more discrete therapy management, and N'Vision® Clinician Programmer Software allowing effective patient management. *Reprinted with permission of Medtronic, Inc.© 2006.*

7.1.1.2 Hydraulic medical devices - artificial urethral sphincters

Artificial urethral sphincters are used primarily to treat stress urinary incontinence in male patients due to radical prostatectomy and in female patients due to intrinsic sphincter deficiency with or without hypermobility of the bladder neck or urethra. These devices are also successfully in managing incontinence with other aetiologies [Petrou et al., 2000]. Artificial urethral sphincters simulate normal sphincter function by opening and closing the urethra under patient control.

The AMS 800™ Urinary Control System (American Medical Systems, Inc., Minnetonka, MN, USA) is an implantable, fluid-filled, solid silicone elastomer device. The basic concept of the system was introduced in 1972 (model AMS 721) and over the years a family of artificial urethral sphincters evolved [Hajivassiliou, 1998]. The system consists of an inflatable cuff, a control pump with lower squeezable part and upper hard part containing deactivation button, and a pressure-regulating balloon reservoir attached to each other with kink-resistant tubing. The pump deflates the cuff placed around the bladder neck or urethra by transferring fluid to the pressure-regulated reservoir. Currently, the AMS 800™ Urinary Control System is the most widely used artificial urinary sphincter and more than 20.000 implants worldwide have been performed. Although durable treatment for stress urinary incontinence is feasible, mechanical revisions are frequent due to inherent design problems [Lai et al., 2007; Patki et al., 2006]. In addition, urethral erosion may occur as a consequence of artificial urethral sphincter implantation.

Recently, a novel device with conditional occlusion has been clinically tested and may offer improved outcomes and decreased complication rates [Knight et al., 2006]. This device, known as the Flowsecure® (Barloworld Scientific Ltd, Stone, UK), incorporates many characteristics in common with the AMS 800™ device. However, it also includes a number of innovative features, which aim to overcome some of the disadvantages of the AMS 800™. A detailed description of hydraulic artificial urethral sphincters is provided in Appendix A.4.

7.1.1.3 Mechanical medical devices

Balloons

A novel approach is the Adjustable Continence Therapy (ACT® for female patients) and Prostate Adjustable Continence Therapy (ProACT™ for men) developed by Uromedica, Inc. (Plymouth, MN, USA). The system is a minimally invasive implant designed to treat stress urinary incontinence after other therapies have failed [Hübner and Schlarp, 2005; Trigo-Rocha et al., 2006]. It consists of two balloons placed para-urethrally just beneath the bladder neck. A titanium port, which is placed in the scrotum or labium, is connected to the balloons that allow incremental postoperative adjustments via percutaneous volume changes in the implant, so as to accommodate any individual responses to the altered outlet dynamic.

Stents

Obstruction of the urinary tract can be overcome by insertion of endoprosthetic devices. Urethral and ureteral stents can be used for the treatment of lower and upper urinary tract obstructions, respectively. Urethral stents are inserted into the urethra and mechanically support the duct wall to keep the lumen of the external urethral sphincter open. Ureteral stents are implanted into the ureter to restore urine flow from the kidneys to the bladder. Urethral stents were first developed to treat urethral strictures, but were also proposed as an alternative to sphincterotomies, the primary surgical treatment for patients with detrusor-sphincter dyssynergia. Sphincterotomies are generally irreversible, and can cause haemorrhage, erectile dysfunction, and bladder neck stricture. Encrustation and bacterial biofilm formation limit the long-term use of these stents [Denstedt et al., 2000].

Biodegradable stents are in an early stage of development but hold considerable promise for overcoming many of the limitations of permanent metallic implants [Chew et al., 2006; Tammela and Talja, 2003]. New configurations of bioabsorbable urethral stents are being developed to overcome sudden collapse of the stent in the terminal phase of bioabsorption, urethral scarring, and periurethral fibrosis. Stents for treating urethral strictures in the anterior urethra are mostly bioabsorbed, whereas those in the prostatic urethra biodegrade into small fragments which are washed out with urine.
Urethral and ureteral stents represent clinically successful devices for management of upper and lower urinary tract dysfunction. Although stents do not restore normal control of the urinary tract, their efficacy, simplicity, and potential reversibility makes them an attractive option for people who would otherwise have irreversible surgery.
Several stents are commercially available. Examples of urethral stents are the Memokath® stent (Engineers and Doctors Wallsten Medical A/S, Kvistgaard, Denmark), and the UroLume® stent (American Medical Systems, Minnetonka, MN, USA). The Memokath® stent is a thermo-expandable nickel-titanium alloy stent featuring shape memory. Studies have shown that the use is problematic in detrusor-sphincter dyssynergia and should be regarded as temporary measure [Mehta and Tophill, 2005], whereas it showed good results in ureteric strictures and prostatic obstruction [Armitage et al., 2006]. Examples of ureteral stents are the InLay Optima® Ureteral Stent and Bardex® Double Pigtail Soft Stent (C.R. Bard, Inc., Murray Hill, NJ, USA), and the Polaris™ Loop Ureteral Stent and Percuflex® Stent (Boston Scientific Corp., Natick, MA, USA).

Urethral slings
An alternative treatment option for stress urinary incontinence includes the implantation of a urethral sling. Using minimally invasive techniques, the sling passes beneath the urethra and it is attached to the abdominal wall or pubic bone. The aim of urethral slings is to provide mechanical support addressing hypermobility of the urethra and intrinsic sphincter deficiency without causing obstructive micturition.
Different materials have been used as slings, including synthetic materials and autologous/ allograft/xenograft natural materials [Bent, 2004]. Textile made of synthetic materials offers several advantages compared with autologous, allograft and xenograft materials. The risk of transmission of infectious agents is eliminated with synthetics. In addition, synthetic materials can be modified to create a range of physical properties by varying material, diameter, construction modality (knitted, woven, thermo-annealed), pore size, and texture. Moreover, the use of autologous material requires that patients undergo a harvest procedure increasing costs and patient discomfort. Synthetic materials have the advantage of being highly standardised in their biomechanical properties and biocompatibility, but their success and complication rate depends on the kind and amount of material. Hence, the advantages of synthetic materials have made them the preferred material of choice in sling surgery, although the ideal sling material has yet to be found. Synthetic slings are mostly composed of polypropylene, polytetrafluoroethylene, polyvinylidene fluoride, polyester, or silicone. Currently, polypropylene has become the most widely used sling material and polyvinylidene fluoride may be a promising new material. Numerous slings are commercially available differing in small but potentially important details (see Table 7.1).

Injectable bulking agents
Bulking agents are indicated for patients with a relatively immobile bladder neck. In most cases, the materials are injected through spinal needles using periurethral or transurethral techniques [Bent, 2004]. A number of materials are marketed, including Contigen® (C.R.

Bard, Inc., Covington, GA, USA), Durasphere® *EXP* and Coaptite® (Boston Scientific Corp., Natick, MA, USA), Macroplastique® (Uroplasty, Inc., Minnetonka, MN, USA), and ZUIDEX™ (Q-Med AB, Uppsala, Sweden). Additionally, specific implanter devices have been developed, including the Macroplastique® Implantation System by Uroplasty BV and the IMPLACER™ by Q-Med AB.

Table 7.1 Examples of synthetic urethral slings

Manufacturer	Location	Name of medical device
American Medical Solutions, Inc.	Minnetonka, MN, USA	In-Fast™ Ultra
		InVance™
		Monarc™ Subfacial Hammock
		SPARC™
Boston Scientific Corp.	Natick, MA, USA	Advantage® Transvaginal
		Lynx® Suprapubic
		Obtryx® Transobturator
Coloplast Corp.	Minneapolis, MN, USA	Aris®
C.R. Bard, Inc.	Murray Hill, NJ, USA	Align™
		Avaulta Solo™
		Uretex®
Gynecare (division of Ethicon, Inc.)	Sommerville, NJ, USA	TVT®
		TVT® Obturator
Promedon SA	Córdoba, Argentina	Argus®
		Safyre™
Uroplasty, Inc.	Minnetonka, MN, USA	I-STOP™
Zeppelin Medical Instruments GmbH	Dornbirn, Austria	Remeex®

7.1.1.4 Other types of medical devices

Intraurethral pump

The In-Flow™ Intraurethral Pump developed by SRS Medical Systems, Inc. (Billerica, MA, USA) incorporates a miniature valve and pump that can be inserted into the urethra to control both continence and micturition in women [Madjar et al., 1999]. The device secures itself in the urethra by means of flexible fins that open in the bladder and a flange at the external urethral meatus. The device is controlled by a remote activator that is placed over the pubic area and is magnetically coupled to the pump. Once activated, the turbine actively pumps urine out of the bladder at a rate of 6-12 ml/s until the bladder is empty. The device is easily inserted by a physician and can be removed by patients when necessary. The In-Flow™ Intraurethral Pump is designed to be replaced every month and is unsuitable for chronic use, but successful usage to an average of 90 days has been reported, at which time the device becomes fouled by salt deposits [Madjar et al., 2000].

Catheters

There are two types of urinary catheterization techniques employed clinically: indwelling catheterization and intermittent catheterization. The indwelling urinary catheter, as used today, was developed by F. Foley and first manufactured commercially in 1930s with the fundamentals of the design of the device remaining relatively unchanged since then [Carr, 2000]. It is a hollow, sterile tube that is passed through the urethra, being retained within the bladder by the inflation of a balloon just below the drainage eyelets of the device. However, inherent flaws remain. Complications include urinary tract infection with device-associated blocking encrustations, biofilm formation, and bacterial adherence. Catheter-associated infections can be reduced if the catheter is removed as soon as possible. Patients can be taught to catheterize themselves within a few weeks after a spinal cord lesion. For self-

catheterization good hand function is a prerequisite. Catheterization seldom stands alone. Concomitant drug use can overcome incontinence and recurrent infections.
Several catheters are on the market, for instance the emteva® catheter (Engineers and Doctors Wallsten Medical A/S, Kvistgaard, Denmark), the Silicone Foley Catheter and Silicone Robinson Catheter (Cook Medical, Inc., Bloomington, IN, USA), the Cysto-Care® FolySil® Indwelling Catheter and Self-Cath® Intermittent Catheter (Coloplast Corp., Minneapolis, MN, USA), and the Bardex® Infection Control Foley Catheter (C.R. Bard, Inc., Murray Hill, NJ, USA).

7.1.1.5 Recent research efforts and future devices

Besides the above discussed devices, a large body of research is carried out, both on the development of new approaches and on the improvement of existing technologies. Specific areas of interest are the following:

- Sacral posterior and anterior stimulation: refinement and improvement of the Finetech-Brindley Bladder System (see also section 7.1.1.1)
- Selective activation techniques: leading to a more physiological continuous micturition pattern, occurring at lower intravesical pressures.
- Bionic neurons (BIONs): injectable, wireless intramuscular microstimulators with integrated electrodes, indicated for urinary urge incontinence. In Europe BION3 is market-approved.
- Intraspinal microstimulation: possibility to achieve coordinated micturition through reductions in sphincter pressure and increases in bladder pressure.
- Control inputs to neural prostheses: enabling a closed-loop system which may replace surgical rhizotomy of sacral posterior nerve roots.
- Para-urethral neuromodulation: implantable system for the treatment of refractory interstitial cystitis; the miniaturo™-I device is undergoing clinical investigations for female patients.
- Percutaneous tibial nerve stimulation: alternative method of neuromodulation in the treatment of overactive bladder symptoms; the Urgent® PC Neuromodulation System is available; an implantable subcutaneous stimulation device is under clinical investigation.
- Optical stimulation of neural tissue: possible future alternative to electrical stimulation in certain situations.
- Artificial optical nervous system: future technology leading to e.g. less electromagnetic interference and more stability, resilience and biocompatibility of wiring.
- Urethral neosphincter construction: surgical technique where the gracilis muscle is transposed from the inner thigh to construct an autologous neosphincter in conjunction with electrical stimulation.
- Intradural nerve anastomosis: experimental technique to construct an artificial somatic-central nervous system-autonomic reflex pathway in patients with spinal cord injury and in children with spina bifida.

An elaborate description of the abovementioned developments is provided in Appendix A.5.

7.1.2 Applications in the Netherlands

Some of the above mentioned medical devices, techniques, and surgical procedures are used in hospitals or have been evaluated in clinical investigations. The Departments of Urology of the Radboud University Nijmegen Medical Centre, Medisch Spectrum Twente and Het Roessingh in Enschede, and Erasmus Medical Center Rotterdam have experience with the Finetech-Brindley Bladder System which enables bladder micturition in spinal cord injury

patients. With the Finetech-Brindley Bladder System selective activation of the detrusor and external urethral sphincter is not possible unless advanced stimulation techniques are used. Although the Radboud University Nijmegen Medical Centre performed research with anodal block in acute clinical studies in the 1990s, none of selective activation techniques are being used in a clinical setting in the Netherlands. The InterStim® neuromodulation therapy for the treatment of refractory urinary incontinence is used at the Department of Urology of the University Hospital Maastricht, Erasmus Medical Center Rotterdam, University Medical Center Utrecht, and Radboud University Nijmegen Medical Centre. BIONs for idiopathic refractory detrusor overactivity incontinence in women have been implanted at the Departments of Urology of the Erasmus Medical Center Rotterdam and University Medical Center Utrecht. An ongoing clinical investigation at the Department of Urology of the Radboud University Nijmegen Medical Centre focuses on an implantable system for tibial nerve stimulation for the treatment of patients with an overactive bladder. Previously, a clinical investigation was conducted with percutaneous tibial nerve stimulation in Nijmegen. The implantation of the miniaturo™-I device in female patients with urge incontinence has been performed at the Department of Urology of the University Medical Center Utrecht. One of the leading centres worldwide regarding dynamic graciloplasty is the Department of Surgery of the University Hospital Maastricht. However, dynamic graciloplasty for urethral neosphincter construction has been abandoned.

7.2 Cell/tissue-based approach for function recovery

Gastrointestinal segments are commonly used as tissues for bladder replacement or repair. However, gastrointestinal tissues are designed to absorb specific solutes, whereas bladder tissue is designed to store urine with minimal solute transport across the urothelium. When gastrointestinal tissue is in contact with the urinary tract, many complications may ensue, e.g. infection, metabolic disturbances, urolithiasis, perforation, increased mucus production and malignancy [McDougal, 1992; Soergel et al., 2004]. Because of the problems encountered with the use of gastrointestinal segments, numerous investigators are attempting to develop a tissue engineered bladder that approaches the mechanical and functional characteristics of the natural bladder.

7.2.1 State of development

The most common cell-based approaches currently under development for bladder repair or replacement are aimed at the following: recovery of the urethral sphincter, treatment of vesicoureteral reflux, and recovery or replacement of the bladder wall.

Recovery of the urethral sphincter

Stress urinary incontinence may occur in case of intrinsic sphincter deficiency, which means that the urethral sphincter (muscles to control the flow of urine from the urinary bladder) does not properly function. Cell-based therapies resulting in a stable and functional treatment of this deficiency have been proposed. One of the suggested approaches for the recovery of the external urethral sphincter of people suffering from incontinence is injecting adult stem cells with the potential to differentiate in muscle cells in the sphincter or bladder neck. Adult stem cells may be derived from striated muscles, fat tissue or as mesenchymal cells from bone marrow [Yiou et al., 2003]. These cells can differentiate in functionally normal smooth or striated muscle cells and thereby have the potential to increase the urethral closure pressure. Favourable findings of trials in animal models and in vitro results are encouraging to start

clinical trials in humans and hold a promising future for the treatment of urinary incontinence [Adamiak and Rechberger, 2005].
Another reported approach to support the function of the external urethral sphincter is the injection of cultured chrondocytes embedded in alginate or cultured on synthetic biodegradable polymers just distal to the bladder neck. The chondrocytes are isolated from a cartilage biopsy, expanded in culture and formulated with alginate to form an injectable gel. Bent et al. [2001] have performed a multi-centre clinical trial in the USA in which the use of autologous ear chondrocytes for treatment of intrinsic sphincter deficiency has been evaluated. Thirty-two patients received a single injection just distal to the bladder neck. There was a decrease in incontinence impact scores in all categories. Furthermore, it was concluded that the treatment of intrinsic sphincter deficiency with autologous chondrocytes is safe, effective, and durable with 50% of patients being 'dry' 12 months after one injection. Twenty-six of 32 patients that showed an effect at three months after the injection maintained the effect at the 12-month visit.

Treatment of vesicoureteral reflux
Vesicoureteral reflux is an abnormal movement of urine from the bladder into ureters or kidneys. Urine normally travels from the kidneys via the ureters to the bladder. In vesicoureteral reflux the direction of urine flow is reversed (retrograde). Vesicoureteral reflux is most commonly diagnosed in infancy and childhood after the patient has a urinary tract infection (Figure 7.2).

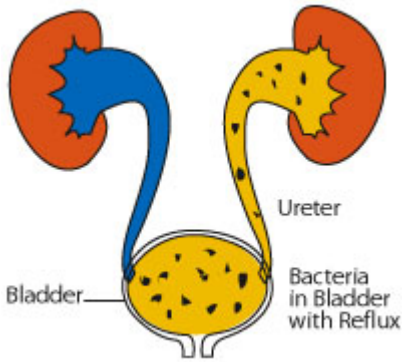

Figure 7.2 Urinary system with vesicoureteral reflux *[www.e-radiography.net]*.

Previous approaches to the endoscopic correction of vesicoureteral reflux are based on the injection of foreign bulking substances around the ureter opening to create a valve function and stop urine from flowing back up the ureter. However, concerns are raised regarding safety and long-term efficacy. A cell-based approach might result in an effective and long-term effect. Diamond and Caldamone [1999] have performed a clinical trial using transurethral injection of autologous chondrocytes to correct vesicoureteral reflux in children. A total of 29 children (46 ureters) with vesicoureteral reflux were treated at two sites. A cartilage biopsy was obtained from each child and chondrocytes were grown in culture for six weeks. Patients then returned for transurethral injection of chondrocytes into the bladder trigone (where the ureters enter the bladder) to correct the reflux. Ultrasound was performed one month and radionuclide cystography was done three months postoperatively to confirm

reflux resolution. When reflux persisted, repeat treatment with stored chondrocytes was offered. The results revealed that initial chondrocyte injection corrected reflux in 26 of the 46 ureters (57%), while secondary injection was successful in 12 of 19 (63%). Overall reflux was corrected in 38 of the 46 ureters (83%) and in 24 of the 29 patients (83%), without the occurrence of significant complications. In conclusion, transurethral injection of autologous chondrocytes to correct vesicoureteral reflux in children appears to be an effective and safe technique that holds promise for treating this congenital abnormality in a minimally invasive fashion.

Recovery or replacement of the bladder wall

The replacement of the bladder wall is the most examined tissue engineering approach for the bladder. The bladder wall is the main part of the bladder and forms the storage vessel for urine. The urothelial layer and the muscle layer are the two layers on which reported cell therapies are focusing. The urothelium acts as a permeability barrier, protecting underlying tissues against noxious urine components, while also stretching to accommodate urine pressures. The muscle layer facilitates the normal expansion-contraction cycles. The cell therapies aim at the replacement of either one or both of these layers. The source of the cells can be both homologous (from the bladder) and non-homologous. Concerning non-homologous cell sources, cultured keratinocytes obtained from skin biopsies have been reported to be used for the replacement of the urothelial layer [Brehmer et al., 2006]. For the non-homologous replacement of the muscle layer, many cell sources have been used including human adipose derived stem cells [Jack et al., 2005], skeletal muscle-derived stem cells [Long et al., 2006; Lu et al., 2005], bone marrow derived stem cells [Chung et al., 2005] and bone marrow stromal cells [Zhang et al., 2005].

However, most research approaches comprise the use of homologous cells in which both the urothelial layer and muscle layer are engineered. Although also the construction of only the urothelial layer [Campodonico et al., 2004] or only the muscle layer [Danielsson et al., 2006] by using bladder derived cells have been reported, this results in a less functional artificial organ as compared to a construct composed of both layers. In order to construct a double layered tissue-engineered bladder, cells derived from a full-thickness biopsy from the patient's diseased bladder are used. In the laboratory, both urothelial cells and muscle cells are isolated from this biopsy. The isolated cells are then culture expanded up to sufficient cell numbers and seeded onto a scaffold material that is shaped like a bladder. The exterior surface is hereby coated with the cultured smooth muscle cells, whereas the interior surface is coated with the urothelial cells to mimic the normal structure of a bladder.

The supporting matrix is ideally composed of an artificial or natural material that allows cell attachment and promotes cell proliferation. This allows the delivery of large cell numbers to specific sites with high loading efficiency. Furthermore, the ideal biomaterial should be biodegradable and biocompatible to support the replacement of the supporting matrix by growing bladder tissue without inflammation. In case the matrix is a natural material, incorporated bioactive factors (e.g., cell adhesion peptides, growth factors) are known to play a role in the cell behaviour of the applied cells. Frequently used natural scaffold materials are small intestinal submucosa (SIS) and collagen sheets. Synthetic materials that are frequently used include polyglycolic acid and copolymers of poly L-lactic acid and poly DL-lactide-coglycolide.

After the cells have formed a layer onto the matrix, the cell-coated matrix is implanted. The implanted cells primarily survive by diffusion of nutrients which is feasible due to the short distances between the implanted cells and the surrounding vascularised tissue at the implantation site. The support matrix should provide mechanical support against the *in vivo* forces that should be maintained during tissue development of the implanted construct. In

time, the cell-coated matrix becomes vascularised, the implanted cells proliferate and the tissue-engineered bladder integrates and restores the function of the bladder. Ultimately the biodegradable scaffold dissolves and is eliminated from the body leaving a functioning bladder made of the patient's own newly regenerated tissue. This principle is illustrated in Figure 7.3. Currently, Tengion, Inc. (East Norriton, PA, USA) is involved in the development of tissue-engineered bladders.

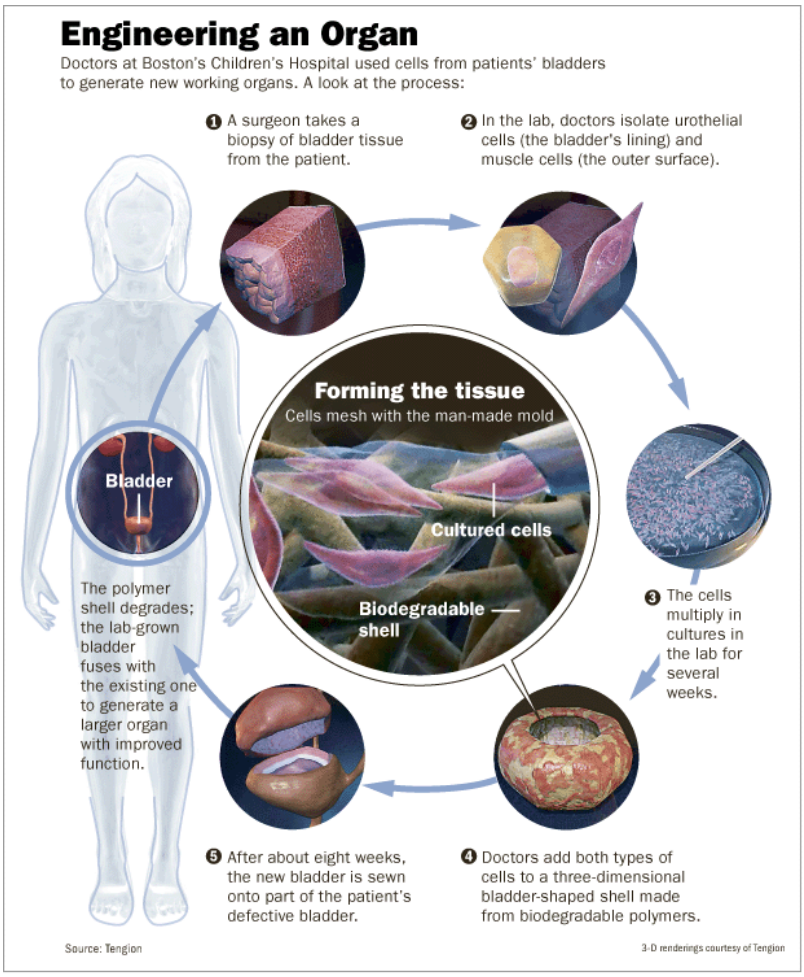

Figure 7.3 Tissue engineering approach using homologous cells. *Reproduced with permission of Tengion.*

This latter described tissue engineering approach has been tested in the clinic in the USA by Atala et al. [2006]. Seven patients with myelomeningocele (spina bifida), aged 4–19 years, with high-pressure or poorly compliant bladders, were identified as candidates for cystoplasty (reconstruction bladder by using intestinal segments). A bladder biopsy was obtained from each patient. Urothelial and muscle cells were grown in culture, and seeded on a biodegradable bladder-shaped scaffold made of collagen (homologous decellularised bladder submucosa), or a composite of collagen and polyglycolic acid. About 7 weeks after the biopsy, the autologous engineered bladder constructs were used for reconstruction and implanted either with or without an omental wrap (peritoneum).
Serial urodynamics, cystograms, ultrasounds, bladder biopsies, and serum analyses were done with a follow-up range of 22–61 months (mean 46 months). Post-operatively, the volume and compliance increase was greatest in the composite engineered bladders with an omental

wrap. This is thought to be due to the enhanced survival and growth of the implanted cells by the rich blood supply obtained from the omental wrap. No metabolic consequences were noted, urinary calculi did not form, mucus production was normal, and renal function was preserved. The engineered bladder biopsies showed an adequate structural architecture and phenotype. As in patients undergoing augmentation cystoplasty (enlargement of the bladder by grafting, e.g. intestine or stomach), all patients were on intermittent catheterization preoperatively due to abnormal innervation caused by their myelodysplasia. Nevertheless, they no longer had to worry about constant incontinence and they faced less danger of kidney failure. The need to use catheters should be different in patients with preoperatively normal innervated bladders, such as patients whose bladder has been largely destroyed by cancer or damaged by an infection or injury. Therefore, a wider variety of patients with defective bladders should be included in clinical trials in the future. In conclusion, the clinical trial has demonstrated that engineered bladder tissue, created with autologous cells seeded on collagen-polyglycolic acid scaffolds, and wrapped in omentum after implantation, can be used in patients who need cystoplasty. As stated Atala et al. [2006], additional studies will be needed before this procedure can be used widely. On the website of Tengion, Inc. it is mentioned that the company is repeating both the preclinical and clinical work, collaboratively with the FDA. Upon successful development and regulatory approval of this product, adults and children suffering from bladder impairment or failure due to congenital disorders, trauma, cancer and other diseases will have alternative treatments potentially offering them better outcomes and improved safety.

7.2.2 Applications in the Netherlands

An umbrella organization in the Netherlands concerning urology is the Dutch Urological Association (Nederlandse Vereniging van Urologie (NVU)) [nvu.artsennet.nl]. The NVU is a specialised medical association for urologists. The aim of the association is to support the development of urology and to serve the interests of all members. The main investigators and specialists of the major hospitals in the Netherlands are involved in this organisation. One of the leading groups involved in the engineering of the bladder is the Department of Urology at the Radboud University Nijmegen Medical Centre. Although no cell-based therapies are used yet, the group has evaluated the use of a collagen-based biomatrix of small intestinal submucosa (SIS) in comparison to a biochemically reconstructed collagen matrix for bladder tissue regeneration. Rabbits underwent partial cystectomy and cystoplasty with either the SIS patch graft or the collagen biomatrix. The grafts of the regenerated bladder wall were harvested at different intervals and tissue regeneration was evaluated. The results of the SIS and biochemically defined biomatrix grafts were comparable. Both matrices show good epithelialisation and ingrowth of smooth muscle cells. Both biomatrices show considerable encrustation, which appears to disappear in time. In conclusion, it appears that the rabbit model is suitable for studying bladder tissue regeneration and is an easy model to use [Nuininga et al., 2004]. In the future, this model will most probably be used to test cell-based tissue engineering approaches. Currently, they are optimising the cell culture process of rabbit urothelial cells and muscle cells.

Another model used by this research group concerns a foetal bladder wall regeneration model in sheep to study different treatments for bladder exstrophy (congenital anomaly in which the bladder is split and situated in the abdominal wall). The model comprises the creation of a bladder extrophy-like lesion in a foetal lamb after 79 days gestation. Subsequently, a dual layer of bladder graft material is sutured in the bladder wall. After further development of the foetus up to 140 days' gestation, the lamb is macroscopically and histologically evaluated for the regeneration of the bladder. Currently, collagen type I biomatrices are tested by using this

model. However, future plans include the testing of tissue-engineered constructs using collagen based substrates and cultured urothelial and muscle cells.
Prof. Feitz is also the coordinator of a European program for soft tissue engineering for congenital birth defects in children (EuroSTEC): from 'biomatrix - cell interaction - model system' to clinical trials. This program has started in January 2007 and forms a strategic combination of basic molecular, cellular and tissue structure scientific knowledge together with clinical paediatric patient care and surgical experience. During the program modern tissue engineering approaches will be developed to treat children with structural disorders present at birth, such as spina bifida, urogenital defects, gastroschisis, diaphragmatic hernia and oesophageal atresia. Close collaboration between different tissue engineering researchers, biomatrix designers and small medical enterprises and a translational route through in vitro and animal experiments should finally lead to early clinical trials. Tailor-made 'smart' biomatrices (scaffolds) will be prepared using natural scaffold molecules and/or man-made polymers, and will be substituted with regulatory molecules such as growth factors and glycosaminoglycans. Furthermore, a variety of cells, including fibroblasts, muscle cells and urothelial/epithelial cells will be cultured in vitro and seeded into these biomatrices. These materials will be implanted using animal models for major congenital birth defects (as discussed above) and evaluated for their capacity to regenerate the correct tissues, including the bladder [Feitz, 2006].
Furthermore, Boevé (secretary of the NVU) has stated that the Sofia Children Hospital had worked on a tissue engineered bladder. However, since the research in the USA was further developed, it was decided to cancel the development of a tissue engineered bladder in The Netherlands [www.zibb.nl/gezondheidszorg]. To our knowledge, no other research and clinical trials focused on a cell-based approach are currently ongoing in The Netherlands.

7.3 Conclusion

Several implantable medical device and (surgical) techniques are available for the treatment of bladder dysfunction. Some of these devices and techniques have proven to be successful, most notably sacral root stimulation, sacral nerve neuromodulation, and artificial urethral sphincters. Far-reaching surgical procedures (e.g., rhizotomy), technical failures (in case of artificial urethral sphincters), and the lack of selective activation must be overcome before these implantable medical devices can gain more widespread use. Many details regarding these techniques have yet to be elucidated. On the horizon are new and emerging technologies (e.g., BIONs, optical stimulation) that could contribute to accomplish improved bladder control. In the Netherlands, academic centres in Nijmegen, Utrecht, Rotterdam and Maastricht are involved in the clinical application of medical devices for the recovery of bladder function.
Several cell-based approaches for bladder repair, reconstruction and replacement are being explored worldwide. These approaches do not comprise the development of the bladder as a whole organ, but are focused on specific parts or diseases of the bladder: I) Recovery of the urethral sphincter, II) Treatment of vesicoureteral reflux III) Recovery or replacement of the bladder wall. Clinical trials have been performed or initiated in all of these groups, although to our knowledge none of these studies are currently performed in the Netherlands.
Nevertheless, a European program in which the Netherlands is playing a key role and most probably includes the development a cell coated artificial bladder wall has been initiated in January 2007. Although it is difficult to predict, it cannot be excluded that early clinical trials using cell-coated artificial bladders will be initiated in the coming five years.

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8. Bowel

The bowel (or intestine) is part of the gastrointestinal tract which is a long muscular tube of approximately nine metres in length and completes the process of digestion. It can be broadly divided into two different parts: the small bowel and large bowel.
The small bowel begins at the pyloric sphincter at the bottom of the stomach and coils its way through the central and lower aspects of the abdominal cavity and joins the caecum at the ileocaecal valve. The small bowel is divided into three separate segments: the duodenum, jejunum, and ileum. The function of the small bowel is the digestion and absorption of nutrients, and the transportation of chyme by peristaltic movement. Digestion is completed in the small bowel with the aid of enzymes secreted from the liver and the pancreas. The chyme is transported to the large bowel for further digestion and disposal.
The large bowel extends from the ileocaecal valve of the terminal ileum to the anus. It comprises of the caecum, colon, rectum, and the anal canal. The main functions of the large bowel are the absorption of water and ions, the degradation of short-chain fatty acids, and the transport and storage of luminal contents. Although the small bowel absorbs some water, this process is intensified in the large bowel until the familiar consistency of faeces is achieved. The large bowel also houses a variety of bacteria crucial for the synthesis of vitamins, such as vitamin K and some B vitamins. In addition, these bacteria ferment carbohydrates and thus play part in the digestion. The content of the colon is moved down to the terminal part of the colon, known as rectum, by peristaltic waves. The anal canal is the terminal section of the rectum. The main functions of the anorectum are to preserve faecal continence, distinguish between solid, semi-solid faeces or flatus (gas), and act as a conduit from the colon to the anal canal and as a reservoir so that defaecation can be accomplished when it is deemed convenient.
The wall of the bowel consists of four layers. the adventitia, muscularis, submucosa, and mucosa. The adventitia consists of a serous membrane composed of connective tissue and epithelium. The muscularis mostly consists of smooth muscle layers, and the nerve supply called myenteric or Auerbach's plexus. The submucosal layer is highly vascular as it is made up of plexuses of blood and lymph vessels. It also contains the submucous or Meissner's plexus. The mucosa consists of epithelium lining the interior of the bowel, the lamina propria made up of loose connective tissue containing blood and lymph vessels and the muscularis mucosae layer with smooth muscle cells.

Bowel function: peristalsis, defaecation, and continence
The intestinal tract consists of longitudinal and circular smooth muscle layers. Contractions of these two layers in the small intestine assist in mixing the chyme with digestive secretions. In the large bowel the coordinated contraction and relaxation of the longitudinal and circular muscle layers supports colonic propulsion. This action is referred to as peristalsis.
Defaecation is the evacuation of faeces from the rectum through the anus. It is a complex process involving temporal coordination of a variety of muscles, nerves and reflex arcs. As filling of the rectum continues, sensory information ascending to the brain leads to sensation of rectal fullness and the urge to defaecate.
There are two major muscles the faeces has to pass through to exit the body. These muscles are the internal anal sphincter muscle (smooth muscle) and external anal sphincter muscle (skeletal muscle) which encircle the anal canal. The external sphincter muscle assists in

retaining the faeces in the rectum until defaecation is needed. Squeezing the external sphincter muscle eliminates the faeces out of the canal and the rectum relaxes.
The ability to retain and evacuate faeces is also dependent on the muscles of the pelvic floor, which are under voluntary control. Two major muscles are the levator ani muscle and the puborectalis muscle, which need to coordinate properly in order to evacuate faeces from the anal canal.
Continence requires appropriate functioning of the puborectalis muscle and the ability to maintain resting internal anal sphincter tone and external anal sphincter contraction in response to increased intra-abdominal pressure, rectal distension, and rectal contraction. Factors such as faeces consistency, rectal and colonic storage capacity, perception of rectal sensation, and cognitive and behavioural function also play important roles.
The current understanding of the neural control of bowel function in humans is still limited. The complex system of the neural supply to the bowel is both autonomic (sympathetic and parasympathetic) and somatic. Although the enteric nervous system can function independently of the central nervous system, the latter has an important role in coordinating the diverse functions of the enteric nervous system. A detailed description of the current knowledge on the neural control of bowel function is provided in Appendix B.1

Bowel dysfunction
A variety of diseases result in the loss of large sections of the bowel, often leading to dysfunction. Short bowel syndrome is a form of intestinal failure resulting from the loss of more than two thirds of the jejunal part of the intestine. This results in diarrhea, dehydration, malabsorption and progressive malnutrition. Colon resection can be require in diseases like cancer, ischemic injury, dysmotility, inflammatory bowel disease and trauma. It leads to problematic changes in the enterohepatic circulation, microbiology and changes in water and sodium absorption. Also in complete bowel functional disorders may occur.
Intestinal dysmotilities may lead to severe propulsive dysfunction. For instance, hyperactivity of colonic muscles generates abnormal bowel movements, abdominal pain, and disordered defaecation. When intestinal muscles are hypoactive, displacement of luminal contents is too slow resulting in obstruction of the intestine and eventually in delayed colonic emptying. Abnormalities in the frequency of intestinal slow waves are associated with intestinal hypomotility. In addition, uncoupled or dysrhythmic intestinal myoelectrical activity leads to a lack of coordinated peristalsis.
Constipation is common, causing decreased bowel frequency, difficulty in defaecation, abdominal pain, and bloating. It can results from a variety of aetiologies. Classically, functional constipation can be divided into three categories: slow-transit constipation, pelvic floor dysfunction, and constipation predominant irritable bowel syndrome. More than one mechanism may contribute to constipation in a patient (e.g., Lembo and Camilleri [2003]).
Faecal incontinence may be defined as the uncontrolled loss of faeces (solid or liquid) from the large bowel. Incontinence may result from traumatic damage to idiopathic degeneration of the sphincter muscles, spinal injury or other neurological causes. It can also result from bowel contractions whose magnitude exceeds the sphincter pressure (e.g., Wald [2007]).

8.1 Medical device-based approach for function recovery

8.1.1 State of development
The medical device-based approach in intestinal failure is not aimed at the replacement of (parts of) the bowel, but at the support or recovery of bowel function. In this section, an

overview will be given of the state of development concerning electrical medical devices for restoring intestinal motor functions either through activation or inhibition of luminal transit along the intestinal tract. In subsequent paragraphs, an overview of hydraulic and mechanical medical devices will be given. Hydraulic medical devices are used to restore faecal continence. Mechanical medical devices, such as stents, can be used in intestinal obstruction due to malignancy. Approaches, devices, and some surgical techniques that are likely to affect the field in the future are also described.

8.1.1.1 Electrical medical devices for neurostimulation

A brief overview concerning the fundamental principles of neuromuscular electrical stimulation and sophisticated stimulation techniques is given in the Appendix A.1, which applies both to bladder and bowel control.

Sacral nerve root stimulation

Stimulation of sacral anterior nerve roots S2-S3-S4 can be used for the treatment of patients with neurogenic bowel dysfunction, e.g. the initiation of defaecation in spinal cord injury patients. Parasympathetic and somatic nerves that supply the distal colon, rectum, and anal sphincters are all derived from the same sacral spinal roots that are stimulated for bladder micturition using the Finetech-Brindley sacral root stimulator (Finetech Medical Ltd, Welwyn Garden City, UK) (see section 7.1.1.1). Together with clinical observations, it seemed likely that the Finetech-Brindley Bladder System could also be used to induce colorectal motility. However, sacral nerve root stimulation alone initiates defaecation in only a small proportion of patients, and the remaining patients need to use additional medication or manual evacuation [Egon et al., 1998; Van Kerrebroeck et al., 1993]. The implantation of electrodes is usually supplemented by severance of posterior nerve roots S2-S3-S4 (rhizotomy). A detailed description of sacral nerve root stimulation is provided in Appendix B.2.

Sacral nerve stimulation

Sacral nerve stimulation in human was first used in 1981 for the treatment of urinary incontinence. In urology it is commonly referred to as sacral nerve neuromodulation (see section 7.1.1.1). In 1994 it was first used for the treatment of anorectal dysfunctions in patients with idiopathic faecal incontinence, i.e. patients with deficient function of the external anal sphincter and levator ani, but with no structural defect [Matzel et al., 1995]. Since then, sacral nerve stimulation has undergone continuous development. The spectrum of indication has expanded to include for example patients suffering from idiopathic constipation [Kenefick et al., 2002a; Malouf et al., 2002], slow-transit constipation [Dinning et al., 2007], faecal incontinence due to systemic sclerosis [Kenefick et al., 2002b], patients with rectal resection for cancer [Matzel et al., 2002], muscular dystrophy [Buntzen et al., 2004], rectal prolapsed repair [Jarrett et al., 2005b], and neurologic dysfunction [Holzer et al., 2007; Jarrett et al., 2005a]. Although these latter results are also encouraging, current data are too sparse and further investigations are required.

Sacral nerve stimulation is a highly precise technique. Depending on patient needs, several implantable devices are offered by Medtronic Corporation, Minneapolis, MN, USA. A detailed description of sacral nerve stimulation is provided in Appendix B.3.

8.1.1.2 Hydraulic medical devices

Artificial bowel sphincter

The artificial bowel sphincter was adapted from the artificial urinary sphincter AMS 800® (see section 7.1.1.2), which was introduced in 1972 by American Medical Systems, Inc. (Minneapolis, MN, USA). The first experiences of the use of the AMS 800® artificial urinary sphincter for faecal incontinence were published in 1987 [Christiansen and Lorentzen, 1987]. In 1996, it was modified for faecal incontinence, creating a specific artificial bowel sphincter, which is now referred to as the Acticon® Neosphincter [Wong et al., 1996]. It is a totally implantable, fluid-filled, solid silicone elastomer medical device. It consists of an inflatable cuff (mimicking the function of the anal sphincter) placed at the level of the anorectal junction, a pressure-regulating balloon implanted in the suprapubic prevesical space or extraperitoneally in the left iliac fossa, and a subcutaneously positioned control pump in either the soft tissue of the scrotum or the labium major (see Figure 8.1). The three components are connected with kink-resistant tubing. The device remains deactivated until healing is completed, usually within 6-8 weeks.

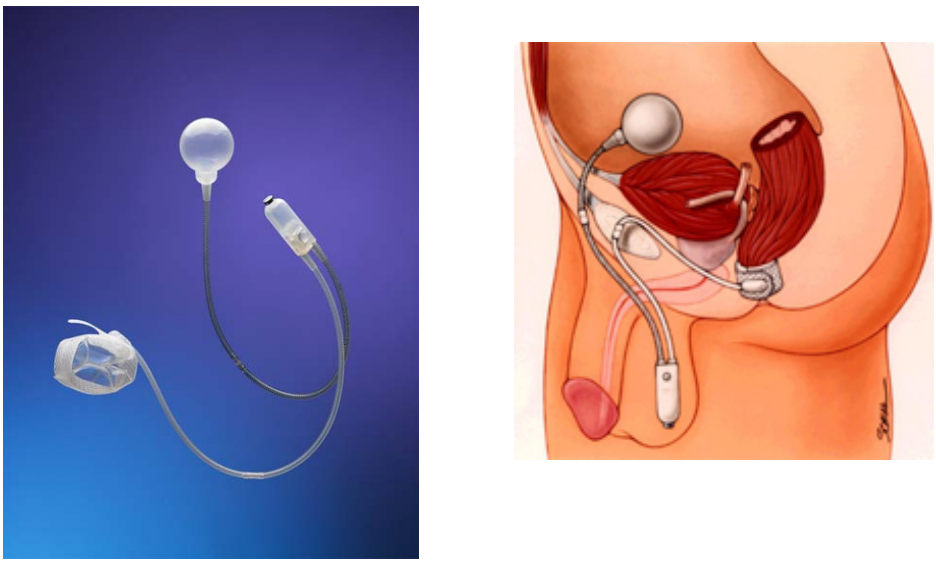

Figure 8.1 Acticon® Neosphincter. The artificial bowel sphincter (on the left) consists of an inflatable cuff (left side), pressure-regulating balloon (middle), and control pump (right side). The artificial bowel sphincter implanted in a male patient (on the right). *Courtesy of American Medical Systems, Inc. (Minnetonka, MN, USA; www.AmericanMedicalSystems.com).*

The device is not without complications and safety issues remain a cause of concern, but its success rate is high. Infection, skin erosion, device malfunction, and pain are common adverse events and many patients require revisional procedures [Devesa et al., 2002]. Once successfully placed, however, the Acticon® Neosphincter results in good functional outcome for most patients, and its functions remains stable for many years [Parker et al., 2003]. The device has not only been shown to improve continence but has actually made patients continent to solid faeces on a daily basis and even to gas.
Neosphincter surgery is technically demanding. Artificial bowel sphincter implantations are performed only sporadically around the world, because of the wide range of strict exclusion criteria and the availability of this surgical option exclusively in large coloproctological centres. It is possible that even ‘high-volume’ colorectal surgical units will not perform more than a few implantations per year in the near future [Belyaev et al., 2006]. When several studies from a single clinical centre over a number of years are compared, it becomes evident to what extent the learning curve for artificial bowel sphincter implantation is related to the outcome, even with a small number of patients per clinical centre. For example, necessary

surgical revisions within this centre started with 65.3% of implanted cases, then dropped to 37.5%, and finally to 12.5% [Lehur et al., 1998; Lehur et al., 2000; Lehur et al., 2002]. Another difficulty independent of surgical excellence is related to the complex pathophysiology of faecal incontinence and the technical limitation characteristic for artificial bowel sphincter implantation. An artificial bowel sphincter causes ischaemia of the gastrointestinal tract at operating pressures required to maintain continence, because of the circular design of its inflatable cuff. Faecal incontinence is far from being just a mechanical problem.

8.1.1.3 Mechanical medical devices

Enteral stents

Enteral stents are being used for palliative treatment and as a bridge to surgery in (acute) obstructive colorectal and duodenal cancers [Khot et al., 2002]. A variety of self-expanding metal stents have been used effectively, including the Wallstent® Colonic & Duodenal Endoprosthesis, Ultraflex™ Precision Colonic Stent System, WallFlex® Colonic & Duodenal Stent (Boston Scientific Corp., Natick, MA, USA), Hanaroostent™ Colo-Rectal & Duodenal Stent (M.I. Tech Co. Ltd., Seoul, South Korea), and Cook® Colonic Z-Stent® (Cook Medical, Inc., Bloomington, IN, USA).

Injectable bulking agents

The use of injectable bulking agents is an evolving treatment for faecal incontinence. Although bulking agents have been used to treat urinary incontinence for many years, their use in colorectal surgery has so far been limited and reports are confined to a small number of pilot studies [Vaizey and Kamm, 2005]. The intention is to facilitate closure of the anal canal by creating a better seal. The agent is injected submucosally to create a bulking effect. It is injected either circumferentially if the internal sphincter muscle is degenerated or fragmented, or at the site where the muscle is deficient to augment the deficient internal sphincter.
A number of materials have been used or are still undergoing clinical investigation to determine their clinical efficacy and long-term safety, including silicone (PTQ™; Uroplasty BV, Geleen, the Netherlands), hydroxyapatite ceramic microspheres (Coaptite®; Boston Scientific Corp., Natick, MA, USA), carbon-coated zirconium oxide beads (Durasphere®; Boston Scientific Corp., Natick, MA, USA), polyacrylamide (Bulkamid™; Contura, Soeborg, Denmark), dextranomer-hyaluronic acid co-polymer (ZUIDEX™; Q-Med AB, Uppsala, Sweden), and biological tissues (autologous fat, porcine dermal collagen).
Injectable bulking agents are rapidly becoming widespread to treat passive faecal incontinence. However, there is little evidence to support this practice. The use of non-autologous agents appears to be relatively safe and does not compromise further therapy, should it be needed. Nevertheless, randomised clinical investigations are definitely required to establish the safety and efficacy of these medical devices [Vaizey and Kamm, 2005].

8.1.1.4 Recent research efforts and future devices

Besides the above discussed devices, a large amount of research activities is carried out, both on the development of new approaches and on the improvement of existing technologies. Specific areas of interest are the following:

- Selective activation of sacral ventral nerve roots: leading to greater efficiency of defaecation using electrical stimulation techniques.
- Intestinal electrical stimulation: modulation of peristalsis via electrical stimulation in case of intestinal motor dysfunctions.

- Percutaneous tibial nerve stimulation: alternative method of neuromodulation in the treatment of faecal incontinence in patients with incomplete spinal cord injury; the Urgent® PC Neuromodulation System (Uroplasty, Inc., Minnetonka, MN, USA) is under clinical investigation.
- German Artificial Sphincter System (GASS): micro-pump driven artificial sphincter for the treatment of major faecal incontinence (proof of principle in vitro).
- Prosthetic anal sphincter: alternative to the artificial bowel sphincter which may overcome the major problem of ischaemic injury at operating pressures required to maintain continence.
- Artificial sphincter using shape memory alloy material: two Ni-Ti shape memory alloy ribbons revealing reversible deformation between flat and arc shape during a temperature cycle induced by foil heaters attached to the ribbons and powered by a transcutaneous energy transmission system.
- Anal neosphincter construction: surgical technique where the gracilis muscle is transposed from the inner thigh to construct an autologous neosphincter in conjunction with electrical stimulation.
- Perineal puborectalis sling operation: recent technique to treat idiopathic faecal incontinence using a specially designed polyester mesh sling.
- Pudendal nerve anastomosis: new approach to treat faecal incontinence by surgical reconstruction of a neo-anus with pudendal nerve anastomosis.

An elaborate description of the abovementioned developments is provided in Appendix B.4.

8.1.2 Applications in the Netherlands

Worldwide, one of the major clinical centres in the field of colorectal surgery the Department of Surgery at the University Hospital Maastricht. Sacral nerve stimulation, artificial bowel sphincter and dynamic graciloplasty are areas of interest.

8.2 Cell/tissue-based approach for function recovery

Solutions with living cells to create artificial bowel segments are all following the classic tissue engineering approach using an extracellular matrix seeded with living cells, mostly accompanied by biomolecules such as growth factors or cell differentiation factors. Tissue engineering of intestinal tissues is considered an emerging field, where significant progress can be expected in the not too distant future [Chen and Beierle, 2004; Penkala and Kim, 2007].

Research in bowel tissue engineering is making use of a variety of different scaffold materials serving as the extracellular matrix. Both synthetic biodegradable materials such as poly-L-lactic acid (PLLA) and polyglycolic acid (PGA) natural materials such as collagen, fibrin and decellularized small intestinal submucosa (SIS) are being employed [Penkala and Kim, 2007]. The use of both autologous and allogenic cell sources is being pursued.

8.2.1 State of development

Although interesting research results have been reported, bowel tissue engineering is still at an early stage where proof-of-concept studies are being performed mostly in rats. Large animal studies are expected in the near future [Penkala and Kim, 2007].

The most promising studies make use of the transplantation of organoid units on polymer scaffolds. Organoid units are multicellular units derived from neonatal rat intestine,

containing a mesenchymal core surrounded by a polarized intestinal epithelium, and contain all of the cell types of a full-thickness intestinal section [Evans et al., 1992].
Small intestine constructs lined with neomucosa and surrounded by smooth muscle cells, which resembled native intestine were developed by Choi et al. [1998]. Other studies where similar constructs were placed in rats having received 75% small intestine resection showed growing (length and diameter) neointestine closely resembling native tissue with a neomucosa that was continuous between native and engineered bowel [Kim et al., 1999a; Kim et al., 1999b; Kaihara et al., 1999a; Kaihara et al., 1999b; Kaihara et al., 2000].
Successful replacement of small intestine with a tissue engineered product after massive small bowel resection was reported in 2004 by Grikscheit et al. [2004].
Tissue engineering of new colon segments has not been researched extensively, yet. Grikscheit et al. [2002, 2003] reported the first instance of tissue engineered colon production from autologous cells. They demonstrated the regeneration of new tissue with mucosal architecture resembling native colon, and found physiological functions using in vitro characterization methods.

8.2.2 Applications in the Netherlands

No clinical applications are currently being performed to our knowledge.

8.3 Conclusion

Recent developments in sacral nerve stimulation, artificial bowel sphincter procedures, and dynamic graciloplasty are considered to be promising. Enthusiasm for any new technique often leads to overemphasis of the outcomes, and early reports are usually good. Outcomes can deteriorate with time and long-term results do not correspond to initial encouraging data such as for instance in case of the artificial bowel sphincter or dynamic graciloplasty. Both methods are technically demanding, with considerable morbidity, and substantial learning curve. Despite these obvious disadvantages, both artificial bowel sphincter and dynamic graciloplasty remain attractive to colorectal surgeons because once successful, they provide outstanding and long-lasting improvement of bowel function and quality of life.
Unfortunately, these procedures require special equipment and their utility is limited because there is high morbidity to consider, which discourage coverage by health care insurers.
Tissue engineering approaches to create novel bowel tissue are currently still at the stage of proof-of-concept in small experimental animals. Promising research is going on for both small and large intestine. However, no clinical studies are expected in the near future.

8.4 References

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Appendix A Additional information - bladder

A.1 Basic principles of neuromuscular electrical stimulation

An electrical stimulator or neural prosthesis delivers trains of electrical charge pulses mimicking to an extent the natural flow of excitation signals generated by the nervous system. The electrical stimulation operation can be modelled with a relatively simple electric circuit: pulse generator, electrodes and tissue. The tissue is an ionic conductor with an impedance of about 10 to 100 Ω, and electrodes are capacitive conductors, whose electrical properties depend on many variables, but their impedance is from 500 to 5 kΩ, and they induce a phase shift of about 10 to 30 degrees.

Basically there are two types of electrode configurations, referred to as monopolar or bipolar stimulation. In the monopolar configuration one or more active electrodes (cathode) are positioned in the vicinity of the structures to be stimulated, while a single common electrode (anode) is positioned relatively distant to the stimulated structures, yet somewhere along the neural pathway to the central nervous system. In the bipolar configuration a cathode and anode are positioned in or near the nervous tissue targeted for stimulation, and the electrical circuit is closed between the electrodes. For some applications, more than two electrodes can be activated in tripolar or quadripolar configurations. Usually implantable systems apply bipolar or tripolar configurations. To prevent leakage of current to neighbouring tissue, tripolar electrodes are preferred [Popovic and Sinkjær, 2000].

The pulse amplitude and duration, output impedance of the pulse generator, and impedance of electrodes determine the electrical charge that will be delivered to neuromuscular structures. Generators are referred to as constant-current or constant-voltage devices. High-output impedance devices will deliver the desired current to the tissue, regardless of the changes in electrode properties up to the voltage capacity available. Constant-current devices are current-regulated stimulators. The electrical charge delivered to the stimulated structure depends on the impedance of electrodes to tissue interface when stimulators that have a low-output impedance (i.e., voltage-regulated stimulators) are applied. This is the reason to use current-regulated stimulators so that the consequences of typical impedance changes can be ignored. If voltage-regulated stimulators are used the voltage may be substantial, however, the actual charge delivered to the tissue may be very small. This may result in pain. In contrast, current-regulated stimulators precisely control the charge delivered to the tissue. However, current-regulated stimulators may cause tissue damage if the surface of the electrode is too small.

The stimulus waveform selected for the excitation process must take into consideration the desired physiological effect (action potential generation), potential damage of the tissue, and potential degeneration of the electrode [McCreery et al., 1995]. In general, the waveform of the pulse is rectangular. A nonrectangular pulse could also be utilised, but the rise time must be sufficiently fast so the nerve membrane does not accommodate and fails to open its ion channels. The stimulus waveform may be unidirectional (monophasic) or bidirectional (biphasic). Biphasic stimulus is recommended for several reasons. For implanted electrodes, the potential for damage to the tissue will be lessened by allowing greater charge injection. Balanced charge stimuli are generally used in which equal charge is delivered in each half-cycle of a biphasic pulse. The electrode that receives the negative stimulus pulse first is the cathode. During the cathodic phase (assuming a monopolar configuration) the charge is delivered first to the active (working) electrode, which is near the depolarisation site. This is important in order to minimize the risk of tissue damage. The anodic pulse is then applied after a short interphase delay following the cathodic pulse. This second pulse will allow the action potential to fully develop, since immediate current reversal may stop firing of a nerve fibre, which is just at its threshold. This prevents the electrode potential developed on the working electrode from corroding due to the application of excessive anodic potentials. The cathodic pulse does not contribute to the corrosion of the electrode [Merrill et al., 2005].

The surface area of the electrode is important for the safety of the stimulation. The geometric surface area may be many times less than the real surface area, depending upon the surface structure of the stimulating electrodes. Large surface areas will diffuse the current and may not effect the excitation desired. A small surface area may result in high charge density and current density. Although absolute safe levels of stimulation have not been established for all electrode types and all stimulation waveforms, safe parameters of stimulation can be estimated once the size of the electrode is known, or vice versa the minimum size of the electrode of the parameters are known [Grill and Mortimer, 2000].

Independent of stimulator design, the typical output parameters that are clinically relevant are pulse amplitude, pulse width, and pulse rate. Either pulse amplitude or pulse width modulation influence the spatial extent of the effective stimulation field as these parameters control excitation for neural tissue, i.e. the number of recruited nerve fibres. In addition, rate modulation plays more of a role in therapy effectiveness based on the temporal pattern of activity delivered to the nervous system, i.e. the summation of exerted force of contracting muscle

fibres. Since timing circuits regulating pulse width and frequency can be easily constructed and controlled with a resolution of ≤1 μs, many electrical engineers use these techniques for the design of stimulators.

A.2 Detailed description of the Finetech-Brindley Bladder System

In the early/mid 1980s sacral nerve root stimulation was reported in patients with spinal cord injury to enable micturition [Brindley et al., 1982; Brindley et al., 1986]. The sacral root stimulation system was developed by Brindley and is currently known as the Finetech-Brindley Bladder System (Finetech Medical Ltd, Welwyn Garden City, UK) or Vocare™ Bladder System (NDI Medical, Inc., Cleveland, OH, USA). Since then some 3000 of these devices have been implanted [Donaldson et al., 2003; Rijkhoff, 2004]. In general, it has provided good results (in some cases for over 15 years) and achieved sustained clinical use in spite of a few drawbacks [Brindley et al., 1986; Brindley, 1994; Creasey et al., 2001; Egon et al., 1998; Kutzenberger et al., 2005; Van Kerrebroeck et al., 1997; Vastenholt et al., 2003]. The two prerequisites of this system are intact preganglionic parasympathetic axons to the bladder and a detrusor that is able to contract.

The implantable components of the Finetech-Brindley Bladder System include tripolar 'book' electrodes, leads, and a passive receiver/pulse generator. The implant has no batteries and the various components are encased in silicone. The external portable control unit consist of an antenna connected to an external transmitter/controller device which allows programming of the stimulation parameters (frequency, duration and amplitude) by a clinician, and provides the power (by radio-frequency coupling between antenna and receiver) for nerve root stimulation. The stimulation programme allows fine variation in pulse width rather than pulse amplitude. Micturition is initiated after the external control unit is activated by the user.

Implantation of the electrodes is technically demanding requiring microsurgical techniques. Two types of electrodes are available, one for intradural implantation on sacral anterior roots [Brindley et al., 1982] and one for extradural implantation on mixed sacral roots [Sauerwein et al., 1990] (see Box A.1 for an explanation of the neuroanatomy of the sacral spinal cord). Intradural electrodes are implanted in the cauda equina of the spinal canal. Surgical access is provided by laminectomy (surgical removal of the posterior arch of a vertebra) of the first two pieces of the sacrum and the fifth and fourth and sometimes the third lumbar vertebra, and subsequent opening of the dural sac. The implantation of electrodes is typically on S2-S3-S4 roots bilaterally. Leads from the electrodes pass through a grommet implanted in the dura and they are passed subcutaneously to the implanted receiver/pulse generator in the anterior abdominal wall. Extradural electrodes are implanted in the spinal canal of the sacrum and surgical access is provided by a laminectomy of the upper part of the sacrum only. Extradural implantation reduces the risk of leakage of cerebrospinal fluid along the leads, the risk of breakage of the leads where they cross the dura from the intra- to the extradural space, and the risk of myelomeningitis. In addition, extradural nerves are more robust than intradural roots, being covered with fibrous epineurium derived from the dura that attaches both types of roots to each other, requiring less dissection than intradural roots [Brindley et al., 1986]. Extradural electrodes are used in individuals in whom arachnoiditis makes separation of the sacral roots impossible. The majority of implants used have intradural electrodes with two, three, or four channels.

The implantation of electrodes is usually supplemented by rhizotomy of the posterior nerve roots S2-S3-S4. In case of intradural implantation, the intradural posterior roots in the cauda equina are transected [Brindley et al., 1982]. With the intradurally approach, however, differentiation between sacral anterior and posterior roots is difficult as anatomical landmarks are ill-defined [Hohenfellner et al., 1992]. In case of extradural implantation, intradural rhizotomy of posterior roots is performed at the site of the conus medullaris usually via a second small laminectomy at the thoracolumbar junction [Sauerwein et al., 1990].

Box A.1 Neuroanatomy of the sacral spinal cord

Most texts of human neuroanatomy contain the classical description of the spinal cord as being composed of medullary segments: 8 cervical, 12 thoracic, 5 lumbar, 5 sacral, and 1 coccygeal [Carpenter et al., 1976]. Each spinal cord segment possesses anterior and posterior roots bilaterally which contain many nerve fibres. Detailed anatomical dissection of the sacral roots from their point of origin in the spinal cord to their exit from the dura has revealed that the anterior root emerges as separate rootlets that fuse gradually to constitute the anterior component of the sacral root. This configuration holds true for the posterior root also. The anterior root emerges from the spinal cord with as many as 20 rootlets from the S1 spinal segment to less than 6 or 7 from at the S4 level. When these rootlets are followed, they group into rootlets bundles. In stead of 15 or 20, they are reduced to 4 or 5 rootlets. Thereafter, the rootlets bundles conjoin and form the anterior root. The sacral roots have an exceptionally long intradural course, in contrast to other spinal roots, which exit from the dura immediately opposite the respective foramen. Intradural root lengths of approximately 20 cm have been reported and varied corresponding to body length [Mersdorf et al., 1993]. Moreover, the anatomical dissection raised reasonable suspicion of significant inter- and intra-individual differences with respect to both the number of spinal segments and anastomoses between various spinal roots. Besides the occasional absence of S5 anterior roots in some human cadavers, coccygeal roots were not present at all. In addition, intradural bridging was frequently seen between adjacent anterior and posterior roots, although connections between posterior roots predominated. No bridging was found between anterior and posterior roots. It is not understood why the sacral spinal cord more often presents anatomical variations than other segments.

Rhizotomy has several substantial advantages, including abolition of detrusor overactivity, resulting in increased bladder capacity and compliance, reduced reflex incontinence, and protection of the upper urinary tracts from ureteric reflux and hydronephrosis [Brindley, 1994]. Rhizotomy also reduces detrusor-sphincter dyssynergia improving urine flow and prevents autonomic dysreflexia arising from distension or contraction of the bladder/bowel. The intervention has certain drawbacks as well, such as abolition of reflex defaecation, and in male reflex erection and reflex ejaculation. However, in many spinal cord injury subjects these reflexes are not adequately functional and can be restored by other techniques. In recent years, patients have often objected to this intervention, not only because of the above mentioned drawbacks, but also because it is irreversible. In addition, researchers in the field of spinal cord repair proclaim means of curing spinal cord injury in the (foreseeable) future.
Generally, good clinical results have been obtained with the Finetech-Brindley Bladder System, but the device does have some drawbacks. Selective activation of the detrusor muscle and the external urethral sphincter is not easily obtained unless sophisticated neural interfaces and stimulation parameters are used (see section 7.1.1.5). In addition to smaller preganglionic parasympathetic fibres innervating the bladder, sacral anterior roots contain large somatic nerve fibres innervating the external urethral sphincter. Since large fibres have lower thresholds of stimulation, excitation of the preganglionic parasympathetic nerve fibres is accompanied by excitation of the somatic fibres, leading to urethral sphincter contraction and urethral occlusion. Thus, while it is possible to activate the sphincter without the bladder, it is difficult to activate the bladder without the sphincter. Curiously, even though the detrusor and urethral sphincter are stimulated simultaneously by the Finetech-Brindley Bladder System, the bladder is successfully emptied. Selective activation is circumvented by utilising the difference in biomechanical characteristics of smooth and striated muscle tissue. The relaxation time of external urethral sphincter is shorter than the relaxation time of the smooth detrusor. When a train of electrical stimuli is applied intermittently for a few seconds (3-9 s), bladder pressure rises with concomitantly closed sphincter. Upon cessation of stimulation, the sphincter relaxes quickly while bladder pressure is transiently maintained during the inter-burst intervals allowing urine to flow. This process, known as post-stimulus voiding, produces effective micturition of the bladder. However, mictutition occurs in spurts at supra-normal intravesical pressure, and when the ‘on’-phase of the stimulus is too long, intravesical pressure can become very high with attendant risk of damage of the upper urinary tract. The sacral nerve roots contain also fibres innervating other pelvic floor muscles, the musculature of the large bowel (see chapter 8) and legs. Movement of the legs during stimulation can be cumbersome for some individuals.

A.3 Detailed description of sacral nerve neuromodulation
In 1981, Tanagho and Schmidt [1982] embarked on a project concerning extradural stimulation of sacral roots to elicit a contraction of the detrusor muscle and posterior rhizotomies to suppress detrusor-sphincter dyssynergia in paraplegic subjects (comparable to Brindley’s programme). Later on, stimulation at low intensity to S3 nerve roots without damaging them (by percutaneous puncture) was applied and an inverse effect was obtained: inhibition of bladder contraction [Tanagho and Schmidt, 1988]. This result gave rise to the term sacral nerve stimulation for neuromodulation or briefly sacral nerve neuromodulation which nowadays is used as therapy for non-neurogenic bladder dysfunction. Techniques for accessing the sacral nerves through the sacral formina were developed making the implant procedure faster and less invasive [Schmidt et al., 1990].
Initial device models were the Itrel-I™ and Itrel-II™ pulse generators developed by Medtronic Corp. (Minneapolis, MN, USA). Currently, the InterStim® and InterStim® II (smaller in size) Implantable Neurostimulators are marketed (Figure 7.1). InterStim® was first introduced in the early 1990s and presently more than 25.000 implants worldwide have been performed [Oerlemans and Van Kerrebroeck, 2007]. The implantable system is comprised of a battery-powered electrical stimulator, an extension cable (not for the InterStim® II), and a lead with quadripolar electrodes. The electrode is usually inserted through the sacral S3 foramen to lie next to the S3 spinal nerve. The stimulator is implanted in a subcutaneous pocket either in the abdomen or buttock area. Subsequent adjustments of the stimulation parameters can be accomplished easily and non-invasively with an electronic programming device. The battery can run for about five years and can be replaced during in outpatient procedure. Sacral nerve neuromodulation is only effective in a subset of patients with lower urinary tract dysfunction, therefore all patients are initially evaluated with a percutaneous electrode connected to an external stimulator to access their response to this treatment before permanent implantation [Janknegt et al., 1995]. Pudendal afferent activity mapping has shown that the S1 roots contribute 4%, S2 roots 60.5%, and S3 roots 35.5% of the overall pudendal afferent activity [Huang et al., 1997]. Despite that S2 carries more pudendal afferents, the S3 spinal nerve is the preferential site of lead implantation in conjunction with the InterStim® device. Excitation of S3 causes less undesired excitation of efferent nerve fibres innervating leg

muscles. Sacral nerve neuromodulation applies mild stimulation of pudendal afferent nerve fibres to inhibit the micturition reflex. The commonly used stimulation parameters are: pulse width 210 µs, pulse rate 10-15 pulses/s, pulse amplitude 1-10 V (twice the sensory excitation threshold), and continuous or cyclic stimulation (on-off time 10-2 s). Bladder emptying is achieved either voluntary or by means of intermittent catheterisation [Jezernik et al., 2002].
The number of technical problems and hence the complication rate has been significantly reduced with changes in the hardware and fine tuning of the surgical technique Hardware improvements concern a new type of battery and the introduction of a new tined lead [Spinelli et al., 2003a; Spinelli et al., 2003b]. The tined lead electrode has four sets of tines, which allow proper self-fixation and no additional fascial anchoring. Surgical advancements, such as superior buttock placement, minimally invasive approach to percutaneous placement of the tined lead electrode under local anaesthesia have led to an increase in patient comfort and a reduction in time needed to perform the procedure [Scheepens et al., 2001; Spinelli et al., 2003a]. Medium-term and long-term outcomes are promising and demonstrate that sacral nerve neuromodulation is safe and effective [Dasgupta et al., 2004; Elhilali et al., 2005; Siegel et al., 2000; Van Voskuilen et al., 2006; Van Voskuilen et al., 2007]. Since the initial implantations, sacral nerve neuromodulation for the treatment of non-neurogenic bladder dysfunction has been well studied [Bosch and Groen, 2000; Starkman et al., 2007; Sutherland et al., 2007]. Inconsistent degrees of success have been obtained in small scale studies with neurogenic patients who represent a clinical challenge for whom only major surgical options are available [Bosch and Groen, 1996; Chartier-Kastler et al., 2000; Chartier-Kastler et al., 2001; Hohenfellner et al., 2001; Kirkham et al., 2002]. The results suggest that the success of neuromodulatory techniques, at least for individuals with spinal cord injury, may depend on the completeness of the injury as well as the specific locations of the electrodes. In addition, it is suggested that intact spino-bulbo-spinal pathways contribute to the positive effects of sacral nerve neuromodulation [Schurch et al., 2003].

A.4 Detailed description of hydraulic artificial urethral sphincters

Artificial urethral sphincters are used primarily to treat stress urinary incontinence in male patients due to radical prostatectomy and in female patients due to intrinsic sphincter deficiency with or without hypermobility of the bladder neck or urethra. These devices are also successfully in managing incontinence with other aetiologies [Petrou et al., 2000]. In spinal cord injury subjects artificial urethral sphincters are not commonly used, but they can be useful in people with lesions leading to a flaccid bladder and urethral sphincter. Artificial urethral sphincters simulate normal sphincter function by opening and closing the urethra under patient control. The AMS 800™ Urinary Control System (American Medical Systems, Inc., Minnetonka, MN, USA) is an implantable, fluid-filled, solid silicone elastomer device. The basic concept of the system was introduced in 1972 (model AMS 721) and over the years a family of artificial urethral sphincters evolved [Hajivassiliou, 1998]. The system consists of an inflatable cuff, a control pump with lower squeezable part and upper hard part containing deactivation button, and a pressure-regulating balloon reservoir attached to each other with kink-resistant tubing. The pump deflates the cuff placed around the bladder neck or urethra by transferring fluid to the pressure-regulated reservoir. The balloon reservoir, which is usually implanted in the preperitoneal retropubic space, is designed to accommodate a volume of fluid that can provide for a range of preset pressures. The device can be implanted surgically via abdominal or vaginal routes, but higher infections rates have been reported with the vaginal approach. The control pump is implanted in a dartos pouch in the scrotum or into the labia majora. In male patients the cuff encircles the bulbous urethra, and the bladder neck or proximal urethra in female patients. The device system is implanted in its deactivated state, i.e. cuff empty and deactivation button deactivated. After 2-6 weeks to allow for tissue healing, the device is activated non-surgically for the first time by sharply squeezing the soft part of the pump. The cuff closes in approximately 3-5 minutes. Further pumping of the bulb moves fluid into the reservoir and empties the cuff, thus opening the urethra. The cuff closes automatically after several minutes when micturition is completed. The unit can be deactivated by pressing the deactivation button on the hard part of the pump before the cuff refills. Currently, the AMS 800™ Urinary Control System is the most widely used artificial urinary sphincter and more than 20.000 implants worldwide have been performed. Although durable treatment for stress urinary incontinence is feasible, mechanical revisions are frequent due to inherent design problems [Lai et al., 2007; Patki et al., 2006]. In addition, urethral erosion may occur as a consequence of artificial urethral sphincter implantation.
Recently, a novel device with conditional occlusion has been clinically tested and may offer improved outcomes and decreased complication rates [Knight et al., 2006]. This device, known as the Flowsecure® (Barloworld Scientific Ltd, Stone, UK), incorporates many characteristics in common with the AMS 800™ device. However, it also includes a number of innovative features, which aim to overcome some of the disadvantages of the AMS 800™. The components of the Flowsecure® are: a urethral occlusion cuff, pressure-regulating balloon, stress relief balloon, and a pump assembly unit with self-sealing port for in situ pressure adjustments. The premoulded, adjustable circular urethral occlusion cuff minimises creasing and potential stress fractures to reduce the possibility of leaking. The stress relief balloon provides low resting occlusion pressure and transmits additional

pressure to the cuff during periods of increased abdominal pressure, e.g. during coughing or laughing. The pressure-regulating balloon is identical to the stress relief balloon but determines the operating pressure of the device. The regulating pressure is adjustable and can be altered by the injection or withdrawal of filling solution with a hypodermic needle from the device in situ through the self-sealing port in the base of the pump assembly unit. This procedure can be repeated a several times to optimise continence. Moreover, it does not require anaesthesia and it confers a major advantage over the AMS 800™ device of which the pressure can only be adjusted by reoperation to exchange pressure balloons.

A.5 Recent research efforts and future devices

Sacral posterior and anterior stimulation

Worldwide the Finetech-Brindley Bladder System has been successfully used now in more than 2500 patients without major changes in hardware and implant technique [Rijkhoff, 2004]. The device may also assist with the evacuation of the bowel (see section 8.1.1.1) and promote penile erection in some male. Despite its success, two issues exist, that if overcome, could help improve the acceptance and performance of the device: posterior rhizotomy and functional but non-physiological micturition patterns. Rather than abandoning stimulation of sacral roots, because of these limitations, several techniques are being developed to address these issues. Recent research efforts aimed at combining neuromodulation (to increase bladder continence) with neurostimulation (to achieve bladder micturition) using posterior and anterior sacral root stimulation [Kirkham et al., 2002]. The objective was to develop a system that triggers neuromodulation and inhibits the bladder only when needed, i.e. when the onset of bladder contraction is observed. Neuromodulation with small pulse widths successfully inhibited detrusor overactivity and increased bladder capacity. However, intermittent stimulation with larger pulse widths to induce micturition was unsuccessful, in spite of large increases in intravesical pressure. Detrusor-sphincter dyssygernia prevented complete emptying; less than 50% of the bladder volume was voided. The posterior and anterior root stimulation system will only be successful when efficient micturition can be achieved. The concept of this approach might be an important next step in the development of sacral root stimulation in that neurogenic detrusor overactivity, previously abolished by posterior rhizotomy, can be eliminated by posterior root neuromodulation. However, detrusor-sphincter dyssynergia, also previously abolished by posterior rhizotomy, prevents micturition of the bladder in this system. Currently, implantation without rhizotomy in patients with severe detrusor-sphincter dyssynergia is not advised. Clinical use of the posterior and anterior root stimulation system will require additional techniques, e.g. selective activation and improved electrode design. The ‘book’ electrodes of the current system are rather bulky and there is a high demand on miniaturisation with regard to minimally invasive surgery and the risk of laminectomy procedures.

Selective activation techniques

Other ways to improve bladder function is focussed on higher selectivity of stimulation, i.e. the detrusor is selectively activated and the urethral sphincter is prevented from contracting. When selective activation techniques (see Box A.2) are adapted for use in implanted systems, the techniques may allow a more physiological continuous micturition pattern and micturition will occur at lower intravesical pressures.

The application of anodal block using a sacral anterior root stimulator in an animal model has been described by Brindley and Craggs [1980], who were able to produce high intravesical pressure. However, they did not report if the technique could achieve successful micturition and they did not succeed in making anodal block work well enough in humans for regular use [Brindley et al., 1982]. Recently, anodal block has been examined in modelling studies to determine stimulation parameters [Rijkhoff et al., 1994a; Vuckovic et al., 2004]. Moreover, animal studies [Bhadra et al., 2002; Bhadra et al., 2006; Fang and Mortimer, 1991; Grünewald et al., 1998; Koldewijn et al., 1994a; Rijkhoff et al., 1994b; Vuckovic and Rijkhoff, 2004] and intra-operative human studies [Rijkhoff et al., 1997; Rijkhoff et al., 1998] have demonstrated large decreases in urethral sphincter activity while producing bladder contractions. Several issues remain with anodal block however. An implantable stimulator capable of producing the pulse waveforms generally required for anodal block is under development and has not yet been tested clinically [Bugbee et al., 2001]. Although anodal blocking allows selective activation of the bladder, posterior rhizotomy may still be required to allow micturition.

Another technique to prevent external urethral sphincter activation with sacral root stimulators uses high-frequency stimulation to block action potential propagation in somatic nerve fibres. Selectivity is achieved by performing stimulation of nerve roots with a signal composed of two distinctive trains of current pulses. Both an acute study [Shaker et al., 1998] and chronic implant studies [Abdel-Gawad et al., 2001; Boyer et al., 2000] in a canine model have demonstrated that high-frequency, low-amplitude biphasic rectangular pulses block urethral sphincter efferent activity while superimposing low-frequency, high-amplitude pulses activate parasympathetic preganglionic efferents which generate detrusor contractions.

In the canine model successful micturition with this stimulation paradigm has been achieved without rhizotomy of posterior roots. Abandoning the necessity of posterior rhizotomy is of major importance as it could contribute

to preservation of erectile and anal sphincter functions. An implantable stimulator that can generate stimuli composed of two different bipolar current pulses with high-frequency capability has been proposed [Boyer et al., 2000; Robin et al., 1998]. A miniaturised full custom version of the implantable stimulator including a feedback loop to monitor the events surrounding the electrode-nerve interface is under development [Donfack et al., 2000]. Recently, the above mentioned high-frequency stimulation paradigm has also been shown effective in humans [Johnston et al., 2005].

Box A.2 Advanced techniques for selective activation of peripheral nerve

Anodal block
Basically, electrical stimulation of small-diameter myelinated nerve fibres also results in activation of larger nerve fibres because large nerve fibres require lower pulse amplitude for activation compared with small nerve fibres. Therefore, decreasing pulse amplitude derecruits smaller nerve fibres first. However, a selective block of large nerve fibres is possible because they need a lower current to be blocked than smaller nerve fibres. Selective activation can be obtained by a combination of cathodic excitation of both large- and small-diameter nerve fibres, and anodal blocking, distal to the excitation point, of the propagation of the evoked action potential in large nerve fibres. Anodal block takes advantage of the fact that nerve fibres close to the anode are locally hyperpolarised by anodal current. If the cell membrane is hyperpolarised adequately (i.e., sufficient hyperpolarisation is maintained for a sufficient duration), action potentials cannot pass the hyperpolarised zone and are interrupted. Thus, anodal block is not a collision block but an arrest of action potentials. When the technique of anodal block is used, an abruptly ending pulse, such as the rectangular pulse, might cause an unintended induction of an action potential under the anode referred to as anodic break excitation [Van den Honert and Mortimer, 1981]. This can be avoided by using a quasitrapezoidal-shaped current pulse which is characterised by an exponentially decaying lagging edge on the trailing phase of the pulse [Fang and Mortimer, 1991].

High-frequency stimulation block
High-frequency stimulation has been shown to block action potential propagation. Nerve conduction can be blocked using current-controlled rectangular monophasic [Solomonow et al., 1983], rectangular biphasic-shaped pulses [Sawan et al., 1996], or continuous sinusoidal pulse waveforms [Kilgore and Bhadra, 2004]. The mechanism of high-frequency block is not known yet and reported results appear to be contradictory in many cases. In particular, it is not clear whether high-frequency block is due to a fatigue mechanism or to a true conduction block in the cell membrane maintaining nerve and motor endplate in a refractory period, preventing muscle fibres from contracting. The first practical application of high-frequency block was utilised to achieve normal recruitment order of nerve fibre diameter (i.e., smallest fibres first followed by larger fibres) in the sciatic nerve of the cat [Solomonow et al., 1983]. A broad frequency range was evaluated and 600 Hz was identified as the optimum block frequency. However, the applied monophasic stimulation is known to be damaging both the nerve tissue and the electrode, and therefore it is unlikely that these stimulation parameters can be used in chronic clinical applications. In another attempt, biphasic current pulses were used rather than monophasic pulses and proper micturition by blocking unwanted urethral sphincter activity was demonstrated [Sawan et al., 1996]. The application of sinusoid waveforms is still controversial regarding safety aspects and the actual current in voltage-controlled stimulation because the important property of electrical stimulation is charge delivery and not the voltage [Rijkhoff, 2003].

Field steering
Nerves fibres in specific fascicles of a peripheral nerve can be selectively activated, particularly if the stimulating contact is in close proximity to the fascicle containing the target nerve fibres. Under implant conditions it is not possible to align a stimulating contact with each particular fascicle in a nerve bundle. Therefore, it would be desirable to be able to tune the electrode by steering the applied electrical field to create a virtual excitation site at or in the target fascicle. Several modelling and experimental studies have been performed to test spatial selectivity in peripheral nerve using nerve cuff electrodes [Deurloo et al., 1998; Goodall et al., 1996; Grill and Mortimer, 1996; Sweeney et al., 1990; Sweeney et al., 1995; Veraart et al., 1993]. In these studies it was concluded that a transverse current improves the spatial selectivity of a longitudinal tripolar electrode configuration (with a central cathode) and an additional steering anode opposite the cathode. Thus, the application of anodic steering current restricts the region of excitation. It can be used to hyperpolarize an undesired nerve fascicle while activating the target fascicle in a peripheral nerve. In contrast, the application of cathodic steering current can be used to increase the excitation level of a fascicle located between two contacts while not activating other nearby fascicles [Tarler and Mortimer, 2004]. A major consideration for this application is manufacturing the electrode/lead assembly. Current designs employ four radial placed contacts, one for a monopolar and three for the tripolar configuration. Four radial placed monopolar electrodes require four independent leads while four radial placed tripolar electrodes will require at least eight or twelve independent leads. Such an assembly containing eight or twelve independent conductors requires high manufacturing skills to construct. Moreover, it remains questionable whether electric field steering with nerve cuff electrodes obtains sufficient spatial selectivity in sacral nerve roots [Roszek et al., 2000].

<u>BIONs</u>
A novel technology providing an alternative approach to relatively large neural prostheses, which design can lead to time consuming surgical procedures as well as technical failures, is based on microstimulators or bionic

neurons (BIONs) [Loeb et al., 2001]. BIONs™ refer to a family of injectable, wireless intramuscular microstimulators with integrated electrodes (Box A.3).
Originally, BIONs were developed by researchers at a consortium of institutions in the USA and manufactured by Advanced Bionics Corp. (Valencia, CA, USA). At present, Boston Scientific Corp. (Natick, MA, USA) is planning to undo its 2004 acquisition of Advanced Bionics, but will retain the BION business. BIONs are based on a 'platform' technology using generic modules which can be configured and applied to a wide range of anatomical sites and physiological functions. One or more of these BIONs can be implanted by percutaneous injection with the aid of a modified 12-gauge Angiocath™ (hypodermic needle) directly into the sites requiring stimulating or sensing channels. It is minimally invasive and a relatively simple procedure similar to a Botox injection. The patient/user is provided with a Personal Trainer™, a compact handheld user interface containing a microcontroller into which the clinician downloads up to three different stimulation programmes and which records the times and durations of each stimulation programme that the patient/user activates. The Personal Trainer™ is manufactured under contract by Aztech Associates, Inc. (Kingston, Ontario, Canada).
Three generations of this technology have been developed. The BION1 and BION2 platforms have applied to treat complications of disuse muscle atrophy and paralysis, for instance in stroke patients [Salter et al., 2004; Weber et al., 2004]. All platforms are designed to stimulate myelinated afferent or efferent axons, typically in peripheral nerves in muscles. They are not used to stimulate muscle fibres directly because muscle fibres are much less excitable and generally require pulse strengths that are not achievable with this technology. In Europe BION3 is market-approved. This device can be implanted adjacent to the pudendal nerve and is indicated for urinary urge incontinence, i.e. idiopathic (arising spontaneously or from an obscure or unknown source) refractory detrusor overactivity incontinence [Bosch, 2005; Groen et al., 2005]. BION3 is programmed and charged by an external coil but can generate stimulation programmes autonomously while drawing energy from an internal, rechargeable lithium battery. It is capable of receiving and transmitting data by modulated radio-frequency telemetry. BION3 weighs only 0.7 g and it has a size of 27×3.3 mm (length×diameter). It can be programmed to produce various preset patterns of stimulation for a few days (depending on the stimulation parameters) before being recharged by the inductive link. The battery life is projected to be five to ten years depending on discharge and recharge cycles. In order to maximize the power efficiency and safety of the BION within the constraints of its unusually small package, the novel electrode-tissue interface system is developed based on special properties of tantalum and iridium.
Currently, a new class of BIONs is under development at the A.E. Mann Foundation for Scientific Research (Santa Clarita, CA, USA). BION4 is a rechargeable, battery-powered, intercommunicating stimulator and sensor. A high data-rate communication protocol allows large numbers of implants to exchange information freely among multiple implanted BIONs and with an external controller. Sensing modalities can be based on bioelectrical recordings (e.g., impedance of tissue, electromyography), acceleration using a microelectromechanical system to sense acceleration of the implant with respect to the gravitational field, or relative position (distance and orientation) of BIONs when one BION is transmitting an radio-frequency signal and other BIONs in the vicinity can record the strength of the detected signal [Lowery et al., 2006].

Box A.3 Design of BIONs

The BION™ device consists of a small cylindrical capsule for the stimulation module with two rigidly mounted electrodes on its end. The electronic subassembly in the capsule is dominated physically by the antenna coil, which consists of about 200 turns of insulated copper wire wound over a cylindrical ferrite form to maximize the capture of energy from the magnetic field. It is wound so as to be self-resonant at the 2 MHz carrier frequency generated by the external primary coil. The cylindrical ferrite form is actually a sandwich of two hemi-cylindrical ferrites glued to a custom integrated circuit mounted on an alumina ceramic printed circuit board. This arrangement maximizes the surface area available for the integrated circuit and provides a stable platform for its wire bonds plus a discrete diode chip and soldered termination of the coil windings.
The printed board circuits are built in wafers. The wafer is laser-drilled in a pattern that becomes the edge-conductive detents at the ends of the printed circuit boards. One end of the wafer makes electromechanical contact with a platinum-iridium washer welded to a tantalum stem of the tantalum electrode. The other end makes contact to a gold-plated Elgiloy® spring that provides an electromechanical connection to the iridium electrode at the other end, via a hollow tantalum feedthrough. The electronics are sealed into the capsule by sliding them into an open-ended capsule, which is formed onto the tantalum electrode, and welding the tubular feedthrough to the glass capillary walls of the capsule.
The most important requirement for the package is to protect the electronic circuitry from the deleterious effects of water. Sophisticated electronic circuitry such as the tuned radio-frequency power and data receiver and the digitally controlled stimulus pulse generator are particularly vulnerable to condensed moisture. There are well-developed sealing techniques and test methods for implantable electronic devices such as pacemakers and cochlear implants, but these devices are much larger than BIONs. Moisture resistance is addressed by incorporating anhydrous magnesium sulphate powder moulded into a small cylinder of silicone elastomer that fits over a spring inside the BION capsule. Small amounts of magnesium sulphate tend to bind a very large volume of water vapour. Hermetic testing reveals that the minimal guaranteed life time for the package is 30 years. The mechanical integrity of the BION package and seals is also tested in several destructive qualification tests. In addition, devices survive five cycles of autoclaving and freezing without loss of hermetic function [Loeb and Richmond, 2001].

Intraspinal microstimulation

Over the last few years intraspinal microstimulation has been investigated as a possible alternative to conventional implantable neural prostheses for peripheral nerve stimulation. Research so far has been focused on experimental studies in animals to elucidate potential mechanisms using electrophysiological tools, neuro-anatomical tools [Carter et al., 1995; Grill et al., 1999; Pikov and McCreery, 2004], and computer modelling [Moffitt and Grill, 2004], and on functional magnetic resonance imaging in humans to localise microstimulation target sites [Stroman et al., 2002]. In addition, electrode development has improved the ability to selectively stimulate specific regions within the spinal cord. Electrode arrays for chronic intraspinal microstimulation use microwire-based electrodes [Mushahwar and Horch, 2000], silicone substrate microelectrodes using photolithographic manufacturing processes [McCreery et al., 2004], or cylindrical multi-electrodes [Snow et al., 2006]. If intraspinal microstimulation is to be clinically useful, clear advantages over sacral root stimulation must be offered, especially since an intraspinal microstimulation implant would likely be at least as difficult to perform as a sacral anterior root stimulator implant. The ability to actively inhibit urethral activity is not possible with conventional sacral root stimulation. The potential value of intraspinal microstimulation lies not in the direct stimulation of bladder efferents, but in the possibility of activating sacral interneurons to achieve coordinated micturition through reductions in sphincter pressure and increases in bladder pressure. Interneurons exist in various regions around the central canal, in the dorsal gray commissure, and in the intermediolateral cell column of the sacral spinal cord [Shefchyk, 2001] and are active during micturition [Buss and Shefchyk, 2003]. Interneurons in the dorsal gray commissure are of particular interest in relation to inhibition of the external urethral sphincter as the interneurons contain inhibitory neurotransmitter, receive direct projections from the pontine micturition centre. In spinal intact animals, microstimulation in the dorsal gray commissure can produce bladder pressure with no change or reduction in urethral pressure [Blok et al., 1998; McCreery et al., 2004] and can generate some micturition when electrodes around the central canal are stimulated [Grill et al., 1999]. The clinical applicability of intraspinal microstimulation remains far in the future.

Control inputs to neural prostheses

Sensing of bladder responses using implanted sensors might be beneficial in individuals with neurogenic bladder dysfunction. The information could be used to detect the onset of passive or active bladder pressure increase. This in turn could be used to trigger an electrical stimulator that would activate a reflex inhibiting the urethral sphincter contraction. Such a closed-loop system may replace surgical rhizotomy of sacral posterior nerve roots.
Artificial sensors often cause problems in a chronic implantation setting [Koldewijn et al., 1994b]. With the advent of methods for long-term electrical interfacing with nerves, however, recording from natural sensors in the human body may become a realistic alternative (Box A.4). Electroneurographic (ENG) recording cuff

electrodes are presently the most viable sensor technology for long-term peripheral nerve interfaces. Although conceptually promising, ENG recordings from afferent fibres are difficult to use in practise due to the overall signal-to-noise ratios typically seen. However, improvements are being made on several fronts, including better nerve-electrode interfaces, and improved signal processing and amplification circuitry (e.g. Donaldson et al. [2003], Hansen et al. [2004]).

Box A.4 Nerve activity as natural sensor

Nerve cuff electrodes are used because they have proven suitable for chronic human implantation and can be used for whole nerve electroneurographic (ENG) signal recording [Hoffer et al., 1996]. These electrodes allow a measure of the electrical activity in the nerve to be recorded directly as potential difference. Unfortunately, due to low current densities travelling within the afferent nerve fibres, these potentials are subject to interference with sources inside the body (primarily from muscle activation potentials) as well as from external sources (electronic devices, mains power, etc.). The ENG signal picked up by a cuff is small, typically in the 1-10 μV range with a power spectrum between 1 kHz and 3 kHz. The electromyographic (EMG) noise originating from nearby muscles are in the low mV range, two or three orders of magnitudes larger than the ENG signal. What makes ENG recordings feasible despite a noisy environment is the fact that the frequency spectra of the EMG and power-line noise are predominantly below 1 kHz. The EMG power spectrum is centred around 250 Hz but reaches up to 3 kHz, whereas power-line noise is located at 50 or 60 Hz. Both sources of noise can be reduced using filtering techniques. Common-mode noise is further reduced by using a balanced tripolar electrode configuration and differential recording amplifiers with high common-mode rejection and high input impedance. In addition, the implantation of an amplifier in close proximity of the nerve cuff and the use of a telemetry system for transcutaneous transmission of recorded ENG signals eliminate noise problems [Hoffer and Kallesøe, 2001].

It has been shown that bladder sensory information can be extracted by recording ENG signals using cuff electrodes placed on the pudendal nerve [Wenzel et al., 2005], pelvic nerve and sacral nerve roots [Jezernik et al., 2000; Jezernik et al., 2001] in animal studies. Changes in intravesical pressure or wall tension are the primary stimulus for activating afferents. Subsequent chronic implants in pigs demonstrated that afferent ENG signals from the bladder can also be recorded with implanted electrodes [Kurstjens et al., 2001]. Intra-operative ENG signals have been recorded acutely in passive [Grill et al., 2000] and active bladder contractions [Kurstjens et al., 2005] in spinal cord injury individuals who underwent implantation of an extradural Finetech-Brindley Bladder System. Despite the promising results, the requirements for fully implanted ENG signal recording systems are far more stringent than for external systems. Implantable systems are not yet commercially available.

Para-urethral neuromodulation

The miniaturo™-I system is an implantable system for the treatment of refractory interstitial cystitis [Nissenkorn and De Jong, 2005]. Interstitial cystitis is disabling chronic condition of the lower urinary tract characterised by bladder, pelvic and perineal pain, urinary frequency and urgency. The miniaturo™-I device was originally developed by BioControl Medical Ltd (Yehud, Israel). Currently, American Medical Systems, Inc. (Minnetonka, MN, USA) is finalising the development of the device.
The device consists of a battery-powered stimulator and a bipolar stimulation lead. Implantation can be done under local anaesthesia and is less demanding and quick. The stimulator is placed in a subcutaneous pocket in the lower abdominal area, with the stimulation lead placed in the pelvic floor adjacent to the urethral sphincter and attached to the stimulator. Currently, the miniaturo™-I device is undergoing clinical investigations for female patients.

Percutaneous tibial nerve stimulation

Percutaneous tibial nerve stimulation represents an alternative method of neuromodulation in the treatment of overactive bladder symptoms [Govier et al., 2001; Van Balken et al., 2001; Vandoninck et al., 2003]. The posterior tibial nerve is chosen as target site because it is, besides the common peroneal nerve, the acupuncture point used to inhibit bladder activity in traditional Chinese medicine. Reductions in incontinence caused by neurogenic lower urinary tract dysfunctions have also been reported in small-scale studies [Amarenco et al., 2003; Andrews and Reynard, 2003]. Centrally, the posterior tibial nerve projects to the sacral spinal cord in the same area where bladder projections are found. These are most probably the areas where the therapy effect of neuromodulation of the bladder takes place.
For percutaneous tibial nerve stimulation a 34-gauge needle is inserted percutaneously near the nerve approximately 5 cm cephalad to the medial malleolus of the ankle and just posterior to the margin of the tibia. A surface electrode is placed on the medial aspect of the ipsilateral calcaneus. The needle and electrode are connected to a low-voltage electrical stimulator. Stimulation sessions last for 30 minutes and are repeated

regularly in an outpatient setting. This will occur over a 10-12 week period, after which the patient's response is evaluated. If the patient is responding well, the patient is entered into a tapering protocol that individualises the frequency of treatments to the patient's personal requirements, i.e. treatment frequency is slowly reduced to minimise the number of visits while still maintaining the beneficial carry-over effects of the therapy. Treatment frequency may range from once per week to once every two months. Given the technical simplicity of this technique and its potential to suppress detrusor overactivity, more substantial data to determine efficacy in larger patient groups and long-term follow-up are needed. Although percutaneous tibial nerve stimulation is minimally invasive, easily applicable and well tolerated, the main disadvantage seems to be the necessity of maintenance treatment as modulation effects are temporary [Van der Pal et al., 2006].
A commercial device, the Urgent® PC Neuromodulation System, is available from Uroplasty, Inc. (Minnetonka, MN, USA). The system is a combination of the Urgent® PC Stimulator and the Urgent® PC Lead Set, formerly known as the Urosurge SANS device. Currently, an implantable subcutaneous stimulation device is under clinical investigation allowing individuals to stimulate themselves at home as frequently as they require. This implantable device will lessen the burden on medical professionals and institutions.

Optical stimulation of neural tissue

A fundamentally different approach to neural stimulation is emerging using solid-state diode-laser technology causing a transient energy deposition in neural tissue directly resulting in an evoked action potential. Optical stimulation (or radiation) has several advantages over electrical stimulation. Pulsed laser light enables contact-free and artefact-free stimulation of discrete population of nerve fibres, and thereby enhances spatial selectivity. Optical systems might be superior to electrical system in applications where avoidance of current spread is crucial. While optical stimulation is unlikely to replace electrical stimulation, the technology has the potential to impact neural stimulation and could be an alternative to electrical stimulation in certain situations [Wells et al., 2007].
Recently, it has been shown as proof-of-principle that axons in the peripheral sciatic nerve [Wells et al., 2005a; Wells et al., 2005b] and the cranial auditory nerve [Izzo et al., 2006] can be stimulated using low-power pulsed infrared-laser light. The underlying mechanism of optical stimulation is unclear, although it may involve photothermal effects evidenced by localised elevations in temperature.
Stimulating nerve fibres using optical radiation might benefit neural stimulators, including neural prostheses. Currently, an infrared nerve stimulator (Capella™, model R-1850) for use in medical research and scientific applications is marketed by Aculight Corporation (Bothell, WA, USA). An implantable system for optical stimulation based on solid-state diode-laser technology will take many years of research and development. Recent advances in optical stimulation are demonstrating that neurotechnology may follow in the footsteps of communications technology and move from electrically-based devices to optically-based devices.

Artificial optical nervous system

Another development is focused on the use of fibre optic cables in conjunction with microcrystal energy converters such as photovoltaic devices. Fibre optic cables may offer an alternative signal and power distribution system where replacing the leads with non-metallic optical fibres could address electromagnetic interference and radiological artefact safety issues. In addition, optical fibres may be more stable, resilient and biocompatible relative to the saline environment of the body compared to metal leads.
In a proof-of-principle demonstration it has been shown that the peripheral sciatic nerve of a frog can be stimulated *in vitro* by a photovoltaic neurostimulator connected to a multimode optical fibre, converting optical activation pulses from an infrared diode-laser light source into an electrical stimulation signal [Song et al., 2007]. Additionally, an integrated cortical neuroprobe is under development where analog electrical signals recorded from the motor cortex by a microelectrode array are extracted as a high data rate digital infrared optical signal, which can be guided into an optical fibre [Patterson et al., 2004; Song et al., 2005]. Eventually, a network of a compact neurostimulator with superfine optical fibre wiring is envisaged to provide an assistive artificial optical nervous system for advanced neural prosthesis, where the fibre optic wiring may eventually allow a direct communication link from the cortex to a distant excitation site. The promise of an artificial optical nervous system (as well as optical stimulation) may take years before it emerges as a viable medical technology.

Urethral neosphincter construction

Dynamic graciloplasty is a surgical technique where the gracilis muscle is transposed from the inner thigh to construct an autologous neosphincter in conjunction with electrical stimulation (Box A.5). The electrical stimulation replaces voluntary activation of the gracilis muscle and is required lifelong. It transforms fast-twitch, fatiguable (type II) muscle fibres into slow-twitch, fatigue-resistant (type I) muscle fibres within a few weeks giving the transposed gracilis muscle properties required to function as a neosphincter providing sufficient urinary outlet resistance to maintain continence. Dynamic graciloplasty can reinforce sphincter function of the

bladder in neurologically impaired patients [Janknegt et al., 1992; Janknegt et al., 1995] as well as in patients with radical prostatectomy [Chancellor et al., 1997].
Dynamic graciloplasty is technically demanding, require a considerable learning experience and should be confined to specialist centres. Despite promising potential of dynamic graciloplasty for the treatment of urinary stress incontinence, the technique has not gained widespread clinical acceptance because poor clinical outcomes are associated with the limited length of the gracilis muscle [Perez-Abadia et al., 2001] and long-term clinical outcomes are disappointing mostly due to atrophy of the urethra and the gracilis muscle [personal communication].

Intradural nerve anastomosis

A new approach for the treatment of a neurogenic bladder is based on the surgical reconstruction of nervous pathways. Intradural nerve anastomosis is an experimental technique to construct an artificial somatic-central nervous system-autonomic reflex pathway in patients with spinal cord injury [Xiao et al., 2003] and in children with spina bifida [Xiao et al., 2005]. A microsurgical anastomosis between anterior roots of lumbar and sacral spinal cord segments seems to restore bladder function up to a certain degree (Box A.6). Unexpectedly, children with spina bifida who gained bladder storage and micturition functions also gained bladder sensory function, maintained by the ability to sense a full bladder and the desire to void. The pre-existing sensory neural network may have been activated by stretch as the detrusor tone and bladder storage function improved. Since spinal cord continuity is not interrupted as in spinal cord injury, the central nervous system at both spinal and supra-spinal levels may have plasticity to accommodate the artificial somatic-autonomic reflex pathway for micturition.

Box A.5 Dynamic graciloplasty for neosphincter construction

The gracilis muscle is the most superficial adductors on the medial side of the thigh. It has a favourable neurovascular entry located along the proximal-medial one-third of the muscle belly. The distal part can be dissected without the risk of nerve innervation disturbances. As the origin of the muscle is located near the perineal part of the bladder outlet, it is technically possible to wrap the distal part around the urethra and the bladder neck. A transection of the gracilis muscle is performed at the most distal part of the tendon proximal to its insertion at the tibial bone. The gracilis muscle is then tunnelled subcutaneously to the perineum and wrapped around the bladder neck or the bulbous urethra using a split sling configuration. The distal end of the gracilis tendon is sutured to the contralateral pubic bone with non-absorbable sutures under moderate tension. After several weeks to secure the tight connection between the tendinous portion and the pubic bone, a pulse generator (Itrel-II™, Itrel-III™, or InterStim® Implantable Neurostimulator; Medtronic Corp., Minneapolis, MN, USA) and intramuscular stimulation electrodes (model SP 5528, 5566, or 4300; Medtronic Corp.) are implanted. The electrodes are sutured into the muscle belly, tunnelled subcutaneously to the lower anterior abdominal wall, and connected to the pulse generator. The amplitude, rate, pulse width, polarity, and duty cycle of the stimulation can be adjusted telemetrically with an external programmer (model 7432; Medtronic Corp.) by a physician. After implantation and before continuous stimulation is applied, the transposed gracilis muscle is trained for several weeks (8-12 weeks) according to a stimulation protocol. Intermittent stimulation starts with a duty cycle of 0.125 s on and 2 s off. The frequency rate is 25 Hz and the voltage is adjusted at the level of contraction perception. The duty cycle is increased every two weeks. After 8 weeks the duty cycle is 100% on. At this moment the frequency rate is decreased to 15 Hz because the contraction of a muscle with predominantly slow-twitch muscle fibres fuses at a lower frequency rate. With an external magnet, the patient can switch the pulse generator on (continence) or off (micturition). This stimulation protocol has been derived from dynamic latissimus cardiomyoplasty [Carpentier and Chachques, 1985] and has some drawbacks. Sequential segmental stimulation and closed-loop control offers improvement and refinement in muscle performance, while algorithm-based function control could allow to perform more complex tasks, e.g. change in posture and lifting heavy objects [Zonnevijlle et al., 2003].

Box A.6 Intradural nerve anastomosis

The surgical procedure involves a hemilaminectomy and a transection of two (or three) anterior roots. The proximal stump of a lumbar anterior root (usually L5) is anastomosed to the distal part of a sacral anterior root (S2 and/or S3) using an absorbable suture. Performing the somatic-autonomic reflex procedure in children with spina bifida is more challenging because of abnormal neuroanatomy. Technically the most important requirement to establish the somatic-autonomic reflex pathway is an undamaged somatic pathway. The microsurgical anastomosis should be tension-free and clearly end-to-end to avoid neuroma and non-functional connections. Patient training and education is also important as patients need help to localise the most sensitive dermatome.
The assumption underlying this technique is that somatic motor axons may be able to regenerate and replace autonomic preganglionic axons, thus, forming functional synapses with postganglionic neurons and, thereby, transferring somatic reflex activity to bladder smooth muscle. This reflex pathway allows patients to initiate micturition. It is assumed that after regeneration and re-innervation of the lower urinary tract a stimulus from the skin of the particular ipsilateral dermatome, in this case of L5, is forwarded to the lower urinary tract and influences its function. With an increased activation of the cutaneous sensory nerves, which may be evoked by pinching the skin or applying electrical stimulation to the skin, the efferent motor neurons of S2 and/or S3 are predominantly activated. A powerful micturition reflex can be initiated after 5 to 15 seconds of cutaneous stimulation. The artificial somatic-central nervous system-autonomic reflex arc becomes functional at about one year postoperatively. Since sacral nerves also innervate the bowel, the somatic-autonomic reflex pathway could function as a skin-central nervous system-bowel reflex for defaecation.

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Appendix B Additional information - bowel

B.1 Neural control of bladder function

The current understanding of the neural control of bowel function in humans is still limited. The neural supply to the bowel is both autonomic and somatic. The distal colon and anorectum are controlled by three sets of peripheral nerves: sympathetic (hypogastric), parasympathetic (pelvic), and somatic (perineal) nerves. The sympathetic postganglionic input is from the Th10-L2 spinal cord segments. The sympathetic nervous system is mainly inhibitory to colonic motility and excitatory to the internal anal sphincter. The parasympathetic nervous system is excitatory to colonic motility as well as inhibitory to the internal anal sphincter. The ascending/transverse colon receives neural input from parasympathetic preganglionic neurons through the vagus nerve and the tranverse/descending colon and anorectum through the sacral nerves S2-S4 via the pelvic plexus. In the small bowel, however, vagal preganglionic neurons innervate only small clusters of select myenteric neurons, which may serve as pattern generators. The superficial perineal nerve (branch of pudendal nerve) provides sensory (afferent) nerve fibres to the perineum, external genitalia as well as anal canal mucosa. Motor (efferent) nerve fibre supply to the pelvic floor and external anal sphincter is from the sacral plexus (S2-S4 level). The levator ani and puborectalis muscles are supplied on both the pelvic and perineal surfaces by direct branches from the nerve roots. The external anal sphincter receives its motor supply from the inferior rectal nerve and the deep perineal nerve [Matzel et al., 1990].

There is also an intrinsic nervous system of the gastrointestinal tract, known as enteric nervous system which closely resembles the central nervous system. Although the enteric nervous system can function independently of the central nervous system, the latter has an important role in coordinating the diverse functions of the enteric nervous system. The enteric nervous system contains sensory neurons, interneurons, and motor neurons. It is well connected to the central neural network of the central nervous system through both sensory and motor pathways of the parasympathetic and sympathetic nervous systems. In the enteric nervous system, the nerve-cell bodies are grouped into small ganglia that are connected by bundles of nerve processes forming two major plexuses: myenteric plexus and submucous plexus. The myenteric plexus provides motor innervation of the longitudinal and circular smooth muscle layers of the bowel and secretomotor innervation to the mucosa. The submucous plexus plays an important role in the secretory control, especially in the small bowel. It can autonomously sense the tension and chemical environment in the bowel and regulate blood vessel tone, motility, secretions, and fluid transport [Goyal and Hirano, 1996].

The basic electrical rhythm governing interstitial contractions is determined by specialised non-neural pacemaker cells called the interstitial cells of Cajal which are innervated by enteric motor neurons (Box 8.1). In the bowel the interstitial cells of Cajal have also a function as conduction pathway for the propagation of electrical slow waves [Ward and Sanders, 2001].

Box B.1 Intestinal pacemakers

One of the peculiar properties of the gastrointestinal tract is the generation of spontaneous rhythmic fluctuations of the resting membrane potential of specialized cells similar to those of the pacemakers cells in the heart. Interstitial cells of Cajal generate slow waves or pacesetter potentials known as intrinsic electrical control activity [Thomson et al., 1998]. The slow wave entrains or synchronises circular muscular contractions along the longitudinal axis of the intestinal tract and directs the propagation of propulsive contractions in the aboral direction. Thus, it paces the myoelectrical activity of the intestine and is referred to as the pacemaker. In addition to the slow-wave frequency, there is another kind of myoelectrical activity in the intestinal tract, known as spike activity or electrical response activity. Spikes can only occur following intestinal slow waves and are superimposed on the slow waves. The normal slow wave frequency is approximately 12 cycles/min in human duodenum and proximal jejunum, with an aboral gradient to about 9 cycles/min in the terminal ileum [Christensen et al., 1966].

In contrast to cardiac pacemakers cells that are organised in nodules or conductive networks, the interstitial cells of Cajal are spread in and between the smooth muscle layers and the distribution of interstitial cells of Cajal differs between regions of the gastrointestinal tract. In the small bowel, interstitial cells of Cajal are located near the myenteric plexus and near the deep muscular plexus [Rumessen et al., 1993a]. In the colon, interstitial cells of Cajal are associated with the myenteric plexus and the submuscular plexus [Rumessen et al., 1993b].

B.2 Sacral nerve root stimulation

Stimulation of sacral anterior nerve roots S2-S3-S4 can be used for the treatment of patients with neurogenic bowel dysfunction, e.g. the initiation of defaecation in spinal cord injury patients. Parasympathetic and somatic nerves that supply the distal colon, rectum, and anal sphincters are all derived from the same sacral spinal roots

that are stimulated for bladder micturition using the Finetech-Brindley sacral root stimulator (Finetech Medical Ltd, Welwyn Garden City, UK) (see section 7.1.1.1). Together with clinical observations, it seemed likely that the Finetech-Brindley Bladder System could also be used to induce colorectal motility. However, sacral nerve root stimulation alone initiates defaecation in only a small proportion of patients, and the remaining patients need to use additional medication or manual evacuation [Egon et al., 1998; Van Kerrebroeck et al., 1993]. The implantation of electrodes is usually supplemented by severance of posterior nerve roots S2-S3-S4 (rhizotomy), i.e. sacral deafferentation.
The first implantations were performed in the late 1970s and early 1980s with much success in managing the neurogenic bladder and bowel [Brindley et al., 1986; MacDonagh et al., 1990; Varma et al., 1986]. However, setting the Finetech-Brindley stimulator for effective defaecation is not simply a matter of using a standard set of stimulation parameters and has to be optimised [MacDonagh et al., 1990; Varma et al., 1986]. When the device is activated, the time taken to defaecate is shortened even if an increased frequency of defaecation is taken into account [Creasey et al., 2001; MacDonagh et al., 1990]. In some patients the device works so well that manual evacuation of faeces, which was formerly needed, has become unnecessary. In addition, abolition of episodes of severe constipation has been reported [Binnie et al., 1991; MacDonagh et al., 1990]. By a process of trial and error, for each patient the most effective nerve root or roots and optimum stimulation parameters are selected. Commonly used stimulation parameters are: pulse width 100-600 μs, pulse rate 8-46 Hz, intermittent stimulation time on-off 10-20 s, and pulse amplitude 10-40 V.
When the Finetech-Brindley stimulator is activated a distinctive pattern of colonic motility is produced [Binnie et al., 1991]. Stimulation affects the motility of distal transverse colon much less than that of the descending colon and suggests relative hold-up in the ascending colon. These effects alter the transit of a faecal bolus in different parts of the colon. The hold-up in the ascending/transverse colon encourage the absorption of water in the ascending colon resulting in more firm stool. Once beyond the splenic flexure (sharp bend where the transverse colon joins the descending colon), the motility encourage compensates for any proximal delay and increase the frequency of defaecation and reduce the total colonic transit time.
During electrical stimulation patients are unable to defaecate. Stimulation elicits a contraction in both rectal smooth muscles and the external anal sphincter causing an increase of anal pressure that is much higher than that of the rectal pressure [MacDonagh et al., 1990; Varma et al., 1986]. After stimulation is stopped, the external anal sphincter relaxes instantaneously and the pressure decreases rapidly to the baseline, but the smooth musculature of the rectum relaxes slowly. This slow decay results in a period of rectum pressure higher than the relaxed anal pressure. A positive recto-anal pressure difference develops and defaecation might occur. The evacuation mode is called post-stimulus defaecation, similar to post-stimulus voiding for the neurogenic bladder.

B.3 Sacral nerve stimulation
Sacral nerve stimulation in human was first used in 1981 for the treatment of urinary incontinence. In urology it is commonly referred to as sacral nerve neuromodulation (see section 7.1.1.1). In 1994 sacral nerve stimulation was first used for the treatment of anorectal dysfunctions in patients with idiopathic faecal incontinence, i.e. patients with deficient function of the external anal sphincter and levator ani, but with no structural defect [Matzel et al., 1995]. The rationale for applying sacral nerve stimulation to faecal incontinence was based on both anatomical considerations [Matzel et al., 1990] and clinical observations of a beneficial effect in faecal incontinence in patients treated for bladder dysfunction [Tanagho and Schmidt, 1988]. Since its first therapeutic application, sacral nerve stimulation has undergone continuous development. The spectrum of indication has expanded to include for example patients suffering from idiopathic constipation [Kenefick et al., 2002a; Malouf et al., 2002], slow-transit constipation [Dinning et al., 2007], faecal incontinence due to systemic sclerosis [Kenefick et al., 2002b], patients with rectal resection for cancer [Matzel et al., 2002], muscular dystrophy [Buntzen et al., 2004], rectal prolapsed repair [Jarrett et al., 2005b], and neurologic dysfunction [Holzer et al., 2007; Jarrett et al., 2005a]. Although these latter results are also encouraging, current data are too sparse and further investigations are required.
Sacral nerve stimulation is a highly precise technique because it comprises of two diagnostic stages followed by a third therapeutic implantation stage (for an overview see Tjandra et al. [2004]). The diagnostic stage are the acute and sub-chronic stages of peripheral nerve evaluation. If the peripheral nerve evaluation shows good results in improving faecal incontinence, a permanent implantable pulse generator known as Itrel-II™ (Medtronic Corp., Minneapolis, MN, USA) or InterStim® (model 3023; Medtronic Corp.) can be offered to patients. The purpose of the peripheral nerve evaluation is to determine the feasibility of implanting an electrode into the sacral formina (acute stage) under local or general anaesthesia and to assess the benefits after a period of stimulation (sub-chronic stage) of sacral nerves. Screening of patients through peripheral nerve evaluation allows preselection of patients who are likely to have good results to sacral nerve stimulation. Acute peripheral nerve evaluation serves to locate the optimal sacral spinal nerve that will elicit contractions of the external anal sphincter and levator ani muscles, thus establishing the integrity of the sacral nerves. Most commonly, the sacral

nerve that is most effective in causing contraction of the external anal sphincter and levator ani muscles is the S3 nerve. Sometimes, more than one temporary electrode is left in place to allow stimulation of different sacral nerves. Occasionally, a bilateral method of stimulation may be required if superior results are obtained compared to unilateral stimulation [Matzel et al., 2002; Rosen et al., 2001]. After acute peripheral nerve evaluation, two technical option can be used for sub-chronic peripheral nerve evaluation: a temporary, percutaneously placed, lead (or multiple leads) or an operative placement of a quadripolar foramen electrode (model 3886; Medtronic Corp.). Recently, a less invasive technique that uses the quadripolar foramen electrode with a modified anchoring device has been proposed and is increasingly used [Spinelli et al., 2003]. Either electrode is connected to a temporary external pulse generator for screening (Medtronic Screener model 3625) with a percutaneous extension cable. Subsequently, the patient is evaluated during a minimum screening period of seven days of sub-chronic peripheral nerve evaluation for improvement of faecal incontinence. At the end of the screening phase, the percutaneously placed temporary test lead is removed and, if successful, a permanent system consisting of electrode, connecting cable and permanent InterStim® pulse generator is implanted. The operatively placed quadripolar foramen electrode is either removed (if unsuccessful) or connected to the implantable pulse generator (if successful), offering the advantage of identical positioning of the electrode during screening and therapeutic stimulation. The pulse generator is placed in a subcutaneous location in the lower abdomen or gluteal area [Scheepens et al., 2001]. On the day after implantation, the pulse generator is activated by telemetry (Medtronic Console Programmer model 7432). The patient is instructed on how to modulate the amplitude of stimulation and deactivate the pulse generator with a hand-held device (Medtronic model 2031) prior to defaecation.
The stimulation parameters are similar to those used in the sub-chronic peripheral nerve evaluation and comparable to those of sacral nerve neuromodulation in urology, sometimes with slight modifications. Commonly used are: pulse width 210 μs, pulse rate 10-25 pulses/s, stimulation time on-off 5-1 s (intermittent stimulation with pulse trains) or continuous stimulation (24 hr/day). Stimulation amplitude (range 1-10 V) is usually adapted to be above the individual patient's perception of muscular contraction or perianal sensation.
The neurophysiologic mechanism of sacral nerve stimulation is poorly understood. Possible mechanisms include stimulation of the motor output from the sacral nerves and pudendal nerve, modulation of local spinal reflexes, and modulation of the autonomic supply to the rectum and pelvic floor as well as spinal tracts to higher centres in the brain.

B.4 Recent research efforts and future devices

Selective activation of sacral ventral nerve roots

Electrical stimulation of sacral anterior nerve roots to evoke defaecation simultaneously activates both the external anal sphincter and rectum [Brindley et al., 1986; MacDonagh et al., 1990; Varma et al., 1986]. Defaecation occurs only in the short period after ceasing stimulation when rectal pressure is higher than anal canal pressure. Efficiency of defaecation might improve if the rectal smooth muscles could be activated selectively, i.e. without simultaneous contraction of the external anal sphincter. Using sophisticated stimulation methods may allow a more neurophysiological activation of the peripheral nervous system (see Box A.2). Anodal block in an acute porcine model enables selective activation of small diameter parasympathetic nerve fibres and blocking of larger diameter somatic motor nerve fibres [Andersen et al., 2005]. As anodal block holds promise in further development of electro-defaecation, chronic animal studies and human studies are needed demonstrate the safety and efficacy of selective rectal activation. In addition, future studies might be able to improve the technique such that both defaecation and micturition can be facilitated.

Intestinal electrical stimulation

Intestinal electrical stimulation as a modality in clinical settings is still in its infancy. Currently, intestinal electrical stimulation is being investigated as a potential treatment modality of intestinal motor dysfunctions. In 1975 the first study with electrical stimulation of the small bowel in an animal model was published [Akwari et al., 1975]. Many animal studies exploring stimulation methods followed in subsequent years (Box B.2). Only few and recent studies have investigated the effects of electrical stimulation of the colon for functional purposes. In 1995 the first study with electrical stimulation of the colon to initiate and support colon emptying in an animal model was published [Hughes et al., 1995].
The encouraging results of the animal models were, however, difficult to demonstrate in humans for nearly three decades. Recently, the feasibility of acute electrical pacing of the duodenum using single-channel long-pulse electrical stimulation in healthy subjects has been reported [Liu et al., 2005]. Duodenal electrical stimulation may have therapeutic potential for the treatment of obesity.
Attempts to modulate colonic peristalsis via electrical stimulation have also been performed in humans. In addition, colonic electrical stimulation could be used to manage colon emptying in colostomy patients who have no voluntary control of evacuation of the colon content. Pacesetter potentials can be entrained in the colon of

healthy subjects and patients with constipation due to colonic intertia using single-channel long-pulse electrical stimulation with intraluminal electrodes placed in the colonic mucosa at different locations, i.e. caecal pole, caecocolonic junction, mid transverse colon, and colosigmoid junction [Shafik et al., 2003b]. When stimulation is applied at a distal location in the colon, the electrically induced pacesetter potentials normalise the increased colonic slow wave activity frequency in patients with irritable bowel syndrome [Shafik et al., 2003a]. Chronic stimulation through electrodes implanted at the colosigmoid junction for six months resulted in normalisation of defaecation, disappearance of abdominal pain, and bloating [Shafik et al., 2004].

Box B.2 Basic principles of intestinal electrical stimulation

Electrical stimulation of the intestinal tract can be achieved via different positioning of the stimulation electrodes [Lin and Chen, 2002]. Most commonly, stimulation electrodes are placed/sutured directly on the serosal surface of the intestinal tract. The advantage of serosal electrodes is the guaranteed contact and direct effect on the target organ. The disadvantage is the invasiveness. A surgical procedure is required using either laparotomy or laparoscopy. Alternatively, electrodes may be placed on the mucosal surface of the intestinal tract. The major disadvantage of this method is that the contact between the stimulation electrode and mucosa is not guaranteed when suction electrodes or intraluminal ring electrodes are used.

Differences in pulse rate (stimulation frequency) evokes distinct physiological responses. A first method consists of electrical stimulation similar to, or slightly higher than, the intrinsic electrical control activity of the stimulated organ also known as slow waves or pacesetter potentials. This approach is aimed to create an artificial slow wave that can entrain and synchronise the (gastro)intestinal electrical activity of the stimulated organ. For the stomach this type of stimulation became known as gastric electrical pacing [Sarna et al., 1976]. A second method involves stimulation at frequencies approximately four to ten times the physiologic rate of the gastric electrical control activity (12-30 cycles/min), and is mainly used for gastric electrical stimulation [Familoni et al., 1997]. A third method consists of electrical stimulation at a frequency of 10-40 Hz. This approach can be used to empty ileal pouches in a canine model [Grundfest-Broniatowski et al., 1988], to create an electrically stimulated smooth muscle neo-anal sphincter in a canine model [Hughes et al., 1995], and to improve colonic transit in a feline model of spinal cord injury [Bruninga et al., 1998]. A fourth method is based on microprocessor-controlled sequential voltage electrical stimulation at a frequency of 50 Hz [Amaris et al., 2002]. This technique utilises four sets of circumferentially implanted electrodes (each set consist of one active and one reference electrode). The approach enables emptying of solid content in the colon. Moreover, it is effective in a chronic canine model of delayed colonic transit [Sanmiguel et al., 2006] and could be a potential treatment for patients with drug-refractory chronic constipation.

On the basis of pulse width, intestinal electrical stimulation can be broadly classified into two categories: long-pulse stimulation in the order of a few ten to hundred milliseconds and short-pulse stimulation in the order of few hundred microseconds. Long-pulse stimulation entrains natural intestinal slow waves of the small bowel with either intraluminal ring electrodes [Lin et al., 2000a] or serosal electrodes [Lin et al., 2000b]. In addition, long-pulse stimulation prevents vasopressin-induced intestinal dysrhythmia, whereas short-pulse intestinal stimulation prevents emetic and nausea symptoms with no effects on intestinal dysrhythmia [Liu et al., 2004]. However, long pulse duration and high stimulation amplitude results in damage of the tissue surrounding the electrodes. In addition, long pulses and high amplitudes lead to high power consumption, which might be a drawback for a fully implantable stimulation system. Thus, the use of the lowest possible values for the stimulation parameters to obtain adequate propulsion is preferable. A novel method of intestinal electrical stimulation, called dual pulse intestinal electrical stimulation, has recently been proposed by combining short pulses and long pulses, i.e. a short pulse is followed by a long pulse [Qi et al., 2007]. This method is capable of improving intestinal dysrhythmia and emetic symptoms but not impaired intestinal motility induced by vasopressin.

Depending on the locations of the stimulation electrodes relative to the anatomical region of the stimulated intestinal organ, electrical stimulation can be used either to accelerate or decelerate transit of luminal content or induce propulsion of luminal content in various segments of the intestinal tract. Single-channel stimulation (i.e., one pair of electrodes) induces either retrograde (backward) or antegrade (forward) entrainment of myoelectrical activity in the intestinal tract. Retrograde stimulation of the distal duodenum delays gastric emptying [Kelly and Code, 1977] and decelerates duodenal transit [Gladen and Kelly, 1980]. Proximal duodenal stimulation near the pylorus does not affect gastric emptying. Likewise, retrograde stimulation of the jejunum decelerates the jejunal transit resulting in enhanced absorption of water, nutrients, and electrolytes [Collin et al., 1978; O'Connell and Kelly, 1987], and reverses the usual aboral direction of transit of liquid chyme in oral direction [Sarr et al., 1981]. Slowing of transit may be beneficial to treat dehydration because of high stomal output after ileostomy, or when segments of small bowel are removed for various pathologic reasons. In contrast, antegrade stimulation of the jejunum drives jejunal content in forward direction [Sarr et al., 1981] and accelerates intestinal transit [Chen and Lin, 2003].

While single-channel stimulation can only accelerate or decelerate intestinal transit, multichannel stimulation or sequential stimulation (i.e., electrode arrays placed serially along an intestinal segment) is able to induce and promote propulsion of solid luminal content of the descending colon [Amaris et al., 2002; Sevcencu et al., 2005a; Sevcencu et al., 2005b]. Stimulation patterns and electrodes were tested in a chronic porcine model to investigate whether methods established in acute animal models are feasible for long term use [Sevcencu et al., 2004]. Experiments to evoke propulsion by sequential stimulation of the small bowel have not been performed yet.

Percutaneous tibial nerve stimulation

Percutaneous tibial nerve stimulation represents an alternative method of neuromodulation in the treatment of faecal incontinence in patients with incomplete spinal cord injury [Mentes et al., 2007]. Percutaneous tibial nerve stimulation is done using the Urgent® PC Neuromodulation System (Uroplasty, Inc., Minnetonka, MN, USA) which consists of a stimulator, a connecting lead, and a surface electrode. A 34-gauge needle with the attached lead is inserted percutaneously near the nerve approximately 5 cm cephalad to the medial malleolus of the ankle and just posterior to the margin of the tibia. A surface electrode is placed on the medial aspect of the ipsilateral calcaneus. The needle and electrode are connected to the low-voltage monopolar stimulator. Stimulation sessions last for 30 minutes and are repeated every other day for four weeks. After this period, percutaneous tibial nerve stimulation is repeated every two months for three times.
Given the technical simplicity of this technique and its potential to neuromodulate rectum and anal sphincters, more substantial data to determine efficacy in larger patient groups and long-term follow-up are needed. Although percutaneous tibial nerve stimulation is minimally invasive, easily applicable and well tolerated, the main disadvantage seems to be the necessity of maintenance treatment as modulation effects are temporary [Van der Pal et al., 2006].

German Artificial Sphincter System (GASS)

A micro-pump driven artificial sphincter has been proposed for the treatment of major faecal incontinence and is currently under development [Schrag et al., 2004]. This experimental high-tech sphincter has less functional components that must be implanted separately. Tissue damage is minimal due to an easy and short surgical implantation technique. The occlusion cuff of the GASS is made of polyurethane. It consists of a support ring including two cuff elements: a fluid reservoir fixed on its outer diameter and a multi-chamber occlusive cuff on the inside diameter. Small filling volumes and low operating pressures reduce the risk of intestinal ischaemia. Both cuffs are interconnected by an integrated piezoelectric silicon micro-pump/valve unit. Bowel tissue can be compressed or relaxed by shifting the fluid between the occlusive cuff and the reservoir cuff. The device has been evaluated around the external sphincter of isolated porcine anal canals *in vitro*.
A novel prototype includes a high-power micro-pump with online pressure measurement inside the occlusive cuff in a single unit. An integrated telemetric interface enables remote control [Schrag et al., 2006]. The prototype is designed for placement around the external sphincter as well as in the area of the anorectal junction. A future prototype will enable the patient to control defaecation voluntarily through a fully implanted subcutaneous pulse generator, similar in size to current cardiac pacemakers, which includes a battery and electronic control and is connected to the GASS by a subcutaneous electrode.

Prosthetic anal sphincter

An alternative to the artificial bowel sphincter is the prosthetic anal sphincter placed around the anorectal junction (lower rectum) via a transabdominal approach [Finlay et al., 2004]. The innovative design is based on the present understanding that a solid can be retained within a tube without the need for a sealed end if an angle or kink is incorporated in the tube. The device consists of a sphincter element, a constant-pressure balloon reservoir, and a control pump. The sphincter component comprises an inflatable linear expander that, when inflated, flattens and angulates the bowel against a soft gel-filled pillow, thus reproducing the action of the puborectalis muscle. The balloon reservoir provides the hydraulic pressure to drive the system. The control pump is implanted in a subcutaneous pouch in the right iliac fossa, where it is operated by the patient. The pump provides the energy to transfer fluid between the sphincter component and the balloon reservoir. Pumping opens the sphincter due to fluid transfer from the sphincter to the reservoir. Pressing the control button on the pump allows fluid to flow back to the sphincter and closes the sphincter.
An advantage of the prosthetic anal sphincter is that it may overcome the major problem of the Acticon® Neosphincter which causes ischaemic injury at operating pressures required to maintain continence. A key feature of the prosthetic anal sphincter is that there are no localised high-pressure areas between the occluding cusps as are created when the Acticon® Neosphincter circular device is applied to the intestinal tract. The prosthetic anal sphincter in turn has also two disadvantages not shared by the artificial bowel sphincter. First, the abdominal approach necessitates surgery of greater magnitude, although in the future it could be performed laparoscopically. Secondly, the prosthetic anal sphincter is placed around the anorectal junction resulting in a short dead space below the implant. This produces a certain degree of postevacuational faecal leakage. The prototype of the prosthetic anal sphincter has been developed by Biosil Ltd (Douglas, Isle of Man, UK).

Artificial sphincter using shape memory alloy material

This novel medical device consists of three components: the artificial sphincter, transcutaneous energy transmission system, and over-heater protector. The concept of the device has been proved in porcine models [Amae et al., 2001; Nishi et al., 2004]. The initial prototype faced some serious complications,

i.e. inflammation, heat burns, and buckling-induced ischaemia. The current prototype has been modified eliminating these risks [Luo et al., 2006].
A nickel-titanium alloy with two-way shape memory effect is the fundamental feature of the device. If shape memory alloy material is subjected to heat treatment of 850 °C for 20 minutes with a flat shape followed by heating at 400 °C for 100 hours with restrained arc shape, these two shapes are memorised in the material. The artificial sphincter consists of two Ni-Ti shape memory alloy ribbons revealing reversible deformation between flat and arc shape during a temperature cycle. The ribbons are connected by hinges and together they form a sandwich occlusion of the bowel at body temperature. Upon heating, phase transformation-associated deformation occurs in the shape memory alloy, resulting in a lumen between two ribbons and enabling the opening of the bowel. Complete deformation of the ribbons leads to a lumen of approximately 20 mm between two ribbons.
For heating the shape memory alloy material, foil heaters are attached to the ribbons. Power transmitted to the heaters is provided by the transcutaneous energy transmission system. Soft silicone elastomer cushions are attached to the shape memory alloy ribbons to avoid heat burns on the intestinal tissues in contact with the artificial sphincter, and to reduce compression injuries. Medical grade silicone elastomer is used for the preparation of thermal insulation, and covers the hinges and the secondary coil of the transcutaneous energy transmission system which is implanted subcutaneously.
To prevent the shape memory alloy ribbons from overheating after the complete deformation, an over-heater protector (temperature sensitive reed switch) is incorporated into the electric circuit of the device. The integrated system is capable to control the surface temperature of the artificial sphincter below 40 °C. In addition, the over-heater protector may extend the duration of artificial sphincter opening. To maintain the open state of the shape memory alloy artificial sphincter without heating (e.g., for a relatively long period postoperatively to promote tissue healing), a specifically designed hinge has been developed enabling both functions of locking and release. With this novel hinge, the first heating opens the artificial sphincter and the open state can be maintained by the hinge after the power is off. To close the artificial sphincter, the second heating deforms the shape memory alloy ribbons slightly and releases the locking mechanism. Subsequently, the shape memory alloy ribbons recover their shape to flat and close the bowel.
The sandwich mechanism for closing the bowel can reduce the risk of buckling-induced ischemia which is reported in hydraulically driven artificial bowel sphincters with a radial squeezing mechanism. A further advantage of this experimental medical device is the reduced occlusion pressure which when too high could contribute to postoperative failures like necrosis and recurrent incontinence due to tissue atrophy.

Anal neosphincter construction

Surgical methods for treating organ dysfunction include dynamic myoplasty. The principle of dynamic myoplasty in colorectal surgery involves the use of biological (autologous) material and medical devices to fashion a neosphincter around the anal canal. Dynamic graciloplasty is a subcategory of dynamic myoplasty. Dynamic graciloplasty was pioneered independently by Baeten et al. [1991] and Williams et al. [1991]. It further developed with slight modifications (Box B.3). The surgical procedure for graciloplasty is technically very demanding and a steep learning curve is experienced with the application. Complications are common and may be serious, including defaecation difficulties, infection, pain, pulse generator displacement, anorectal perforation, and death [Chapman et al., 2002; Ruthmann et al., 2006]. The complex procedure has uncertain functional effectiveness. However, dynamic gracoliplasty remains attractive for colorectal surgeons because once successful, it provides outstanding and long-lasting improvement in carefully selected patients who are not candidates for conservative treatment of faecal incontinence or for whom all previous treatment to restore sphincter function has failed. Dynamic graciloplasty is largely limited to a small number of specialist colorectal centres in which adequate patient volume and surgical experience help assure acceptably low morbidity and satisfactory functional outcomes.

Perineal puborectalis sling operation

The perianal puborectalis sling operation is a recent technique to treat idiopathic faecal incontinence [Yamana et al., 2004]. A specially designed polyester mesh sling (Leeds-Keio mesh™; Yufu Itonaga Co. Ltd., Tokyo, Japan) is introduced along the puborectalis muscle, from the posterior perianal incision running to a small suprapubic incision. Both ends are tied together with moderate tension. The concept of this procedure relies on the theory of the importance of an adequate anorectal angle in the maintenance of faecal continence. If the posterior wall of the anorectal angle is properly sustained and the angle is sharpened by an artificial puborectalis sling, symptoms of faecal incontinence can be reduced. A stretch mesh rather than a non-stretch mesh is considered a physiologic improvement and provides less pressure to the tissue and lower the risk of tissue erosion. There are some possible risks of intra-operative complications, such as rectal injury, bladder or urethral injury, vaginal injury, or intractable bleeding. Although none of these complications have been encountered in this study, some complications have been reported for the tension-free vaginal tape procedure for urinary stress

incontinence. The only postoperative complications for the perianal puborectalis sling procedure are infections and rectal ulcers. If there are minor infections, they can be treated with adequate antibiotics. However, if the perianal or suprapubic wounds open and purulent drainage persists, it is necessary to explant the entire sling. Up to now few patients have been evaluated, although this procedure gives promising initial outcomes.

Box B.3 Dynamic graciloplasty for anal neosphincter reconstruction

Transposed skeletal muscles are most widely used for the creation of a new anal sphincter. The precondition is that this muscle should not be essential for locomotion and/or posture. Furthermore, site, muscle mass, and the position of the neurovascular bundle should allow easy mobilisation and transposition in order to preserve a healthy and functional muscle. Muscle transposition involves the detachment of a suitable muscle which is often the gracilis muscle.
Graciloplasty was first explored without electrical stimulation by Pickrell et al. [1952]. The insertion site (distal end) of the gracilis muscle at the tibia is transected and the muscle is dissected and wrapped around the native anal canal. Dynamic graciloplasty (i.e., transposition plus electrical stimulation) was first reported by Caldwell but problems with the equipment resulted in failure of the technique [Caldwell, 1965; Caldwell, 1967]. The first successful surgical procedure was performed in 1986 by Baeten et al. [1991]. Another substitute to encircle the anal canal is the gluteus maximus muscle [Devesa et al., 1992]. The gluteus maximus is a powerful muscle and is often used naturally as an accessory muscle to continence. Moreover, for posture or walking the gluteus maximus muscle is not important. Therefore, it is a logical choice for the creation of an neo-anal sphincter. However, unlike gracilis, gluteus maximus is an important muscle in daily activities, such as running, climbing stairs and rising from a seated position, and impairment may pose problems for the patient. Thus, the advantage of using the gracilis muscle as a neosphincter is that its loss does not result in functional movement deficit in the limb after transposition. In addition, due to its superficial position and the proximal neurovascular supply, the muscle is easily accessible and can be dissected free distally without damage. If the gracilis muscle length is sufficient, a contralateral attachment of the distal gracilis tendon to the ischial tuberosity is made using an epsilon loop [Williams et al., 1991], a gamma loop [Baeten et al., 1995], or a modified alpha loop, i.e. a split-sling loop – a hole is made in the mid part of the muscle and the distal part is pulled through to make a perfect circular loop [Rosen et al., 1994]. There is, however, some uncertainty as to whether vascularity and/or nerve innervation are endangered by this action. If the length of the gracilis muscle is not long enough, an ipsilateral attachment with an alpha loop can be made [Cavina et al., 1990]. Occasionally both gracilis muscles may be transposed around the anal canal with [Geerdes et al., 1995] or without continuous electrical stimulation [Kumar et al., 1995]. Double dynamic graciloplasty has been applied in two young patients with spina bifida for simultaneous treatment of faecal and urinary incontinence [Geerdes et al., 1997].
Implantation of neural stimulator and the leads usually occurs at the same time as transposition. The gracilis muscle can be stimulated either directly using fixed electrodes on the obturator nerve [Williams et al., 1991] or close to the nerve branches via intramuscular electrodes [Baeten et al., 1991]. The advantage of direct nerve stimulation is the total recruitment of all motor units (a motor unit consists of muscle fibres innervated by one efferent motor axon) with the lowest possible energy demand, resulting in a maximum contraction and a prolonged battery life. However, it is questionable whether maximum contraction is necessary or desirable for reasons of potential ischaemic injury to the muscle or the colon. Leads are tunnelled subcutaneously to a pocket in the lower abdominal wall and connected to the neural stimulator, i.e. an implantable pulse generator (Itrel-II™ or Itrel-III™) or InterStim® Implantable Neurostimulator (Medtronic Corp., Minneapolis, MN, USA). The transposed muscle is then subjected to an electrical stimulation protocol transforming predominantly fast-twitch, fatiguable (type II) muscle fibre into slow-twitch, fatigue-resistant (type I) muscle fibres during a training period for several weeks (8-12 weeks) rendering the gracilis muscle suitable for its new function, i.e. performing a sustained contraction. A normal external anal sphincter has a predominance (80%) of type I muscle fibres. After the electrical stimulation training period the percentage of gracilis type I muscle fibres increases from 43% to 64% [Konsten et al., 1993]. Hence, the gracilis muscle is more fatigue-resistant. After the training period, the patient can switch the mode of operation of the neural stimulator on which causes muscle contraction (patient is continent) and off which causes muscle relaxation (patient can initiate defaecation) with an external magnet. The pulse amplitude, duration, rate, polarity and duty cycle of the stimulation are programmed by a physician.

Pudendal nerve anastomosis

A new approach to treat faecal incontinence is based on the surgical reconstruction of a neo-anus with pudendal nerve anastomosis. Pudendal nerve innervation can transform a neo-sphincter in to an original anal sphincter-like muscle in a canine model [Congilosi et al., 1997; Sato et al., 2000] and in humans [Sato et al., 2005]. A neo-anus is reconstructed by using an inferior portion of the gluteus maximus muscle with a pudendal nerve anastomosis contemporaneously with an abdominoperineal excision of the rectum in patients with low-lying malignancy. The distal end of the nerve innervating the gluteus maximus muscle is anastomosed to the perineural window of the pudendal nerve, where the epineurium is excised over a small area without damaging the funiculus. The neo-sphincter contracts approximately seven months after surgery and the diverting ileostomy (which was initially constructed and planned to be removed after the reinnervation of the pudendal nerve) can be

closed at approximately nine months. A neo-anus with a pudendal nerve anastomosis can be a practical option for selected patients wishing to avoid a stoma after an abdominoperineal excision of the rectum.

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