without the use of a formal scaffold are a powerful example of this method.[15]

A controlled induction of vascular networks by ex-vivo or in-situ strategies will determine when tissue engineering will be incorporated successfully and substantially in clinical practice.

DECELLULARIZATION OF TISSUES TO OBTAIN THE IDEAL SCAFFOLD

Decellularization is an approach that removes resident cells from a tissue or even an organ. This cellular removal can be achieved by physical, chemical and enzymatic methods. The aim is to preserve the native structure and function of the ECM, including its perfusable vasculature. These decellularized matrices can be re-seeded and repopulated with autologous cells to restore morphology and function. The strength of this approach may be the preservation of biomolecular environmental cues that are likely to direct cellular phenotype.[16]

Song and colleagues decellularized cadaveric rat kidneys via detergent-perfusion through the renal artery. Acellular scaffolds were repopulated with human umbilical venous endothelial cells and rat neonatal kidney cells. After seeding, the organs were transferred to a perfusion bioreactor to provide whole-organ culture conditions. Upon transplantation *in vivo,* urine was produced.[17]

The primary aim of such decellularization strategies is to deplete the tissues and organs of their immunogenic elements and to repopulate them with autologous cells. Similar whole-organ decellularization methodologies are tailored for heart, liver, lung and trachea.

CELLS AND STEM CELLS: THE SUBSTRATE

Scaffolds deliver the structural guidance and support for proliferating and migrating cells through their honeycomb structure. They transmit tissue-specific mechanical forces that cue the behavior of cells within it. The appropriate parenchymal-cell populations, are the living building units of new tissues. They are elementary in the generation of 3D-tissues that can be incorporated into the defect.[18]

Cell cultivation allows expansion of the number of cells procured from a small tissue biopsy. However, adult cells may be limited in growth potential, may differentiate too easily, or may rapidly deteriorate by senescence. Stem cells could overcome these problems. Stem cells have the unique ability to self-renew, proliferate indefinitely, and create offspring that differentiates into specialized, mature tissues by asymmetric replication.[19] Nevertheless, stem cells, represent a miscellaneous group of cells, with different levels of differentiation potential. From a clinical reconstructive perspective, it is desirable that they are derived from autologous tissues to avoid immune-mediated reactions.

PLURIPOTENT STEM CELLS FOR USE IN AUTOLOGOUS CONDITIONS

Induced Pluripotent Stem Cells

In 2006, Takahashi and Yamanaka reported how adult human dermal fibroblasts or other human somatic cells could be directly reprogrammed by the introduction of transcription factors.[20] Induced pluripotent stem cells (iPSCs) possess a differentiation potential equal to human embryonic stem cells. In other words, they are capable of differentiating into tissues of all three germ-cell layers given the correct culture conditions, growth factors, and genetic milieu.

As these cells are derived from adult tissues, there are no ethical issues. Another advantage is the possible autologous source of the stem cells, unlike embryonic stem cells. Nevertheless, iPSCs also carry the risk of teratoma formation and genetic instability. At the moment, these cells cannot be efficiently used in clinical, reconstructive settings.[21]

MULTIPOTENT STEM CELLS FOR USE IN AUTOLOGOUS CONDITIONS

Multipotent stem cells are capable of differentiating into multiple cell types. Adult stem cells or organ, specific stem cells can be isolated from several tissue sources, including bone marrow, peripheral blood, skeletal muscle, central nervous system, retina, adipose tissue, and epithelia of skin and digestive tract. Multipotent stem cells from lipoaspirates are extensively investigated and used in a clinical reconstructive context.[22–24]

These cells preferentially generate differentiated cells of the same lineage as their tissue of origin. However, experiments suggest that adult stem cells from various organs could contribute to the regeneration of dissimilar organs with stem cells crossing germ layers. This process is called *transdifferentiation.* This has led to the suggestion of a "stem-cell highway", in which stem cells can transit through the circulation with access to all organs. Homing signals (the "niche" or microenvironment) might influence stem-cell migration to specific sites. Secreted growth and differentiation factors are important extracellular signals that control stem-cell fate.[25,26] Still, vigilance is essential when using adult multipotent stem cells, since ex vivo culture of mesenchymal stem cells promotes chromosomal instability.[27]

UNIPOTENT PROGENITOR CELLS

Blood Outgrowth Endothelial Cells

Lin *et al* and Yoder *et al* characterized late outgrowing endothelial-like colonies of collagen-adherent cells with much higher proliferation capacity than early-outgrowth endothelial progenitor cells (EPCs).[28,29] These cells are named blood-outgrowth endothelial cells (BOECs). BOECs stimulate angiogenesis actively by incorporation into the host vessel, and passively by secretion of proangiogenic growth factors. BOECs can be isolated from umbilical cord blood or adult blood, with the former showing higher proliferation potential but also susceptibility to karyotypic aberrations.[29–31]

In the future, these stem cells, derived from autologous tissues, may play a growing role in cell-based tissue-engineering techniques. Meticulous identification and purification will be essential to ensure predictability and safety in clinical protocols.[31,32]

TISSUE ENGINEERING-BASED CLINICAL APPLICATIONS

In 1997, Cao and colleagues[33] published a report of an athymic mouse with a human ear grown on its back. This

ear consisted of an ear-shaped cartilage structure, implanted subcutaneously. The group seeded the synthetic, biodegradable mold with bovine chondrocytes. This report was viewed worldwide and ignited the fascination for tissue engineering.

Walles performed a Medline search using the term "tissue engineering", which provided 37,000 hits.[34] The majority were proof-of-principle studies, only a few resulted in clinical protocols. For instance, in the context of auricular cartilaginous reconstruction, more than 20 studies have been published in the past decade using cell-based techniques and matrices for ear reconstruction. Clinical engineering however, has only been reported in singular cases. Major shortcomings are resorption of the biological scaffold and collapse of the construct in an *in-vivo* clinical setting.[35]

The progression from bench to bed in other tissue-engineering protocols has also been hampered by many difficulties, mostly related to insufficient vascularization and loss of tissue strength and coherence.

In this section, clinical translational tissue-engineering applications for skin, urethra and bladder, bone, trachea and the cardiovascular system will be discussed in more detail.

SKIN

Reconstitution of skin wounds deeper than the basement membrane requires a restoration of all components to restore thickness, texture, and elasticity, while providing biomechanical resistance to aggressors. Skin substitutes were developed for clinical use to treat extensive wounds such as third-degree burns.[36–38]

Distinct features categorize skin substitutes or skin equivalents:

Natural polymers used in skin tissue-engineering include chitosan, fibrin, gelatin, and hyaluronic acid. Chitosan, a polysaccharide acting as an analog to glycosaminoglycan, is biodegradable and biocompatible. It is used as a hemostatic agent and possesses antibacterial properties. Hyaluronic acid shows excellent biocompatibility. Disadvantages of natural polymers include low mechanical strength, shrinkage and contraction, rapid biodegradability and risk of immunological rejection.

Synthetic materials include nylon, polyglycolic acid and polylactic acid. Due to the ease of fabrication, synthetic polymers such as nylon (Biobrane®, TransCyte®) and polyglycolic acid/polylactic acid (Dermagraft®) are less expensive. However, synthetic materials possess limited tissue compatibility, cellular recognition, and incorporation. These substitutes show their importance mainly in combination with natural polymers.[39–41]

Skin substitutes can also be described according to cell content; *cell-free* (*acellular*) versus *cell-containing* (*cellular*) substitutes:

- *Acellular products* contain matrix only, based on natural or synthetic materials. These products serve as a template for dermal reconstitution, allowing migration of host cells during wound healing.
- *Cellular products* contain living cells such as keratinocytes and/or fibroblasts with or without matrix.

These scaffolds could be applied for short periods to stimulate autologous healing, serving as *biological dressings*. *Cell-free biodegradable scaffolds* may stimulate colonization by autologous cells in the wound environment. *Cell-containing skin substitutes* may provide immediate functional skin replacement. However, in a clinical context, allogenic cells will be rejected.

Skin substitutes can also be divided according to the skin layer they represent: epidermal, dermal or combined (Table 6.1).

EPIDERMAL SUBSTITUTES

Cell engineering[42] emerged from the discovery that human keratinocytes could be cultivated and differentiated on a carpet of irradiated murine 3T3-fibroblasts in a culture dish in appropriate tissue-culture conditions.[43,44] This breakthrough led to the application of cultured autologous keratinocytes on burns in 1981 and later proved to be life-saving in extensive burn wounds with limited donor site for skin grafts. However, graft-take remained below 70% in more than 50% of cases. Even after healing, the reconstituted epidermis remained excessively fragile, mainly due to loss of the rete-peg pattern of the epidermis.[45,46]

In order to restore function and structure of the skin, an epidermal layer will not suffice.

DERMAL SUBSTITUTES

Hence, dermal substitutes were added in a partnership with skin cells. In analogy with the extracellular matrix, the scaffold provides support for dermal fibroblasts and epidermal keratinocytes. Preliminary clinical results on the use of "artificial skin" in 1981 indicated better or equivalent performance of the artificial dermis but poor resistance to infection.[37] The rationale of using an absorbable artificial dermis on deep wounds is to provide a temporary scaffold into which cells can migrate from the wound environment.[47] Therefore, pore-size should be around 100–250 µm. The collagen-glycosaminoglycan architecture of most dermal scaffolds approaches that of normal dermis and completely biodegrades within 30 days. Faster biodegradation might lead to toxic byproducts that impair healing.[48] Major histocompatibility complex (MHC) class-I and class-II antigens are found on cellular components of epidermis and dermis. During the processing of acellular dermis, these MHC-molecules are fully eliminated but reappear following grafting, indicating that cells can invade the scaffold and pore size is sufficient. When the basement membrane of the substitute is preserved, keratinocyte-engraftment is superior.[49]

COMPOUND SUBSTITUTES WITH EPIDERMAL AND DERMAL ELEMENTS

Recently, compound scaffolds were presented featuring dermal matrix-elements in combination with allogenic foreskin keratinocytes and/or fibroblasts (Table 6.1). Thus far, however, none of these cell-containing artificial skin-equivalents has proven to add a significant clinical advantage to healing parameters, despite their highly-corresponding structure and bio-content. This explains why none of the engineered cell-containing skin substitutes has been approved for the European market yet. The cells added are often allogenic and will therefore be rejected