CHAPTER 9

The Next Wave: Tissue Replacement and Organ Replacement

Darren Hickerson, Sita Somara, Almudena Martinez-Fernandez, Chi Lo, Todd Meinecke, Cynthia Wilkins-Port, Julie G. Allickson

Wake Forest School of Medicine, Wake Forest Institute for Regenerative Medicine, Regenerative Medicine Clinical Center, Winston-Salem, NC, United States

INTRODUCTION: BACKGROUND

Some of the most complex medical treatments include replacing or repairing tissues or organs that have been damaged through disease or trauma. Currently, reconstructive surgery is often utilised to improve appearance and function by modifying autologous structures or by implanting synthetic prosthetics. These procedures can be limited, however, by the availability and supply of compatible, functional tissues in the patient. Organ failure is often treated by transplantation of allograft donor organs but can be complicated by organ shortages and immune rejection. To overcome these challenges, the next wave of tissue and organ replacement will require engineered organs and tissues derived from autologous or immune naïve cells grown on biocompatible scaffolds. Tissue engineering combines cells with organic or synthetic biomaterial scaffolds to construct a functional replacement for tissues and organs.

In nature, the capacity to regenerate occurs at varying levels across species. Lower species, such as arthropods, annelids, echinoderms, planaria, amphibians and reptiles, for example, have the ability to actively regenerate limbs, tails and other internal structures. In humans, reparative regeneration of complex structures is limited while other intricate physiological processes, such as the regeneration of blood cells and restoration of the endometrium following each menstrual cycle, occur regularly. Introduction of various growth factors, cells or biomaterials can further promote regeneration potential in humans. Significant work has been done to understand regenerative mechanisms through studying full mass liver regeneration following partial hepatectomy in humans (Michalopoulos, 2010). Factors underlying regenerative processes include the presence of adequate stem or progenitor cells, appropriately differentiated cells and the availability of an appropriate growth factor environment. Regenerative medicine combines these factors to develop processes that recapitulate the environment necessary for engineering tissues or organs with normal functionality.

In this chapter, we present both ongoing and near future development in the field of tissue engineering, beginning with a brief historical review. We address the cellular

Second Generation Cell and Gene-based Therapies
ISBN 978-0-12-812034-7
https://doi.org/10.1016/B978-0-12-812034-7.00009-1

physiology and biomaterial requirements for construction of functional replacement tissues and organs. Product process development and clinical translation are furthermore explored from proof of concept to manufacturing for clinical trials, as well as potential challenges for large-scale production. Requirements for regulatory compliance and trends in commercialisation of tissue-engineered products are also discussed.

Brief History of Tissue Engineering

The first organ to be transplanted in a human was the kidney in 1954, which was transplanted into a twin, thus avoiding immune rejection (Murray, 2005). This was followed by a bone marrow transplantation between twins in 1956 (Thomas et al., 1959). The advent of cyclosporine in the 1980s drastically reduced the risk of organ rejection in nonrelated donor transplants, consequently allowing transplantation to become more routine (Colombo and Ammirati, 2011). Nevertheless, the side effects from long term use of immunosuppressive drugs combined with a shortage of donor organs have motivated the push for technology to supply an alternate source for replacement tissues and organs for therapeutic application. Tissue engineering opens up a promising avenue to address these problems.

Regenerative medicine refers to the process of regenerating body subcomponents in vivo or ex vivo and may require cells, natural or artificial scaffolding materials, growth factors or combinations of all three elements. Bioengineering, synonymously termed tissue engineering, is an interdisciplinary field that applies engineering and life science principles with the goal of potentially restoring or replacing damaged tissues or organs.

In the 1930s, Charles Lindbergh, the aviator, and Alexis Carrell, a Nobel Prize winner in medicine, pooled their expertise in engineering and medicine to build an early heart pump and explore the development of ex vivo bioengineered organs (Badylak and Nerem, 2010). In the 1970s and 1980s, skin grafting became one of the earliest applications of tissue engineering (Badylak and Nerem, 2010; Bell et al., 1979; O'Connor et al., 1981). The term 'tissue engineering' was initially used in a case report of successful keratoprosthesis in 1985 (Wolter and Meyer, 1985). In 1988, a study reported seeding foetal and adult rat and mouse cells onto synthetic scaffolds, reproducing extracellular matrix (ECM) networks and subsequently promoting vascular ingrowth (Vacanti et al., 1988; Vacanti, 1988). The concepts of regenerative medicine and tissue engineering were further defined and developed in the 1990s (Murray, 2005; Thomas et al., 1959; Miller et al., 2012) with numerous constructs successfully produced during this time (Vacanti, 2010). One of the first laboratory-produced tissue-engineered organs came from the laboratory of Anthony Atala implanted in 1999 with work beginning in 1990 (Atala et al., 2006). Atala produced tissue-engineered organs using the patient's own cells as to avoid rejection. One other famous tissue-engineered structure was the ear, generated with a synthetic scaffold shaped like an ear, seeded with bovine chondrocytes and implanted under the dorsal skin of an athymic mouse (Cao et al., 1997). In 2008, a bioengineered trachea

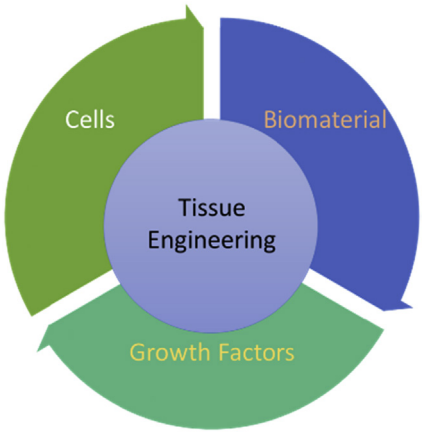

Figure 9.1 ***Basic elements of tissue engineering.*** The three main elements of tissue engineering are cells, biomaterial and growth factors, which are used in different combination to form the final function tissue-engineered product.

that was fully manufactured outside the body by using decellularised donor trachea seeded with autologous epithelial cells, and mesenchymal stem cell–derived chondrocytes were successfully implanted in a patient (Macchiarini et al., 2008; Harding et al., 2013; Union, 2018;Veves et al., 2001; Marston et al., 2003).

Key Considerations in Tissue Engineering Applications

One of the greatest barriers to developing tissue-engineered therapies is a limited understanding of key factors in developmental and progenitor cell biology and the effects of environmental interactions on regeneration potential. The basic elements among all tissue engineering approaches are the cells, biomaterials, biologically active factors and the combination of all these elements (Fig. 9.1).

Understanding the association of these elements for a specific application is paramount for success. The following sections on cells and biomaterials address some of the advance challenges in these areas.

CELLS IN CELLULAR AND TISSUE THERAPIES

Cells provide metabolically active biological components for bioengineered regenerative interventions. On their own or coupled with biomaterial scaffolds, they promote an adaptive microenvironment through secretion of factors, recruitment of endogenous cells and restoration of tissue homoeostasis at the functional level. Consequently, implanted cells must proliferate and differentiate to integrate with the surrounding tissue. Proliferation and differentiation are competing states (Ruijtenberg and van den Heuvel, 2016) with progenitor (stem) cells displaying high-proliferation rates, while

differentiated counterparts tend to divide at a slower pace until they reach cell cycle arrest. This scenario allows for rapid in vitro expansion of progenitor cells prior to implantation and guided preimplant differentiation that facilitates the desired therapeutic outcome. Stem cells are broadly classified based on their origin as embryonic, adult and perinatal stem cells. Each of these categories implies specific characteristics that are advantageous for different regenerative applications.

Embryonic (and Embryonic-Like) Stem Cells

Embryonic stem cells (ESCs) are pluripotent: they have the capacity to differentiate into every cell type of the organism. In vivo, their existence is limited to a very narrow window at the onset of embryonic development, prior to specification and differentiation of tissues. ESCs can be isolated and maintained in vitro for extended periods of time under defined culture conditions and can be directed to differentiate by modifying their culture medium and environment. During the first decade of the 2000s, salient characteristics regarding these naturally occurring cells, including high-proliferative capacity and broad differentiation potential, were reproduced using nuclear reprogramming of adult somatic cells known as *induced pluripotent stem cells* (iPS cells) (Takahashi et al., 2007). This Nobel prize-winning technology opened up new possibilities for utilising stem cells in therapy, by circumventing the ethical and biological concerns attached to use of embryos and by identifying an alternative resource to autologous cells.

Pluripotent stem cells have compelling features including fast, nearly unlimited proliferation potential paired with tremendous differentiating plasticity. These traits render them an invaluable tool for biomedical research (Trounson and DeWitt, 2016). The same properties, however, can hinder their value in clinical applications; cells with a tendency for uncontrolled growth and the capacity to give rise to multiple cell types are difficult to regulate for therapeutic release. Today, only a handful of applications using ESCs have reached clinical trial stage, but mainly for clinical indications associated with the eye, (Clinical Trials, 2014) and the use of ESCs or iPS cells in tissue engineering applications has been limited due to early stages of exploration with these cells.

Adult Stem Cells

Normal physiological growth, maintenance and regeneration of human tissues are supported by pockets of stem cells that are capable of sustaining healthy turnover rates during our life span. This natural capacity to proliferate and differentiate into mature cell types can be leveraged as a therapeutic resource for regenerative purposes. Such is the case for mesenchymal stem cells (MSCs), one of the predominant stem cell types utilised in regenerative medicine and cell-based therapy. The occurrence of MSCs is limited in their natural environment. Once isolated and cultured in vitro, these cells have the potential to differentiate towards multiple cell types. Additionally, when transplanted in vivo, they display extraordinary characteristics such as antiinflammatory, immune-modulatory

and reduced immunogenicity. The ability of MSCs to engraft into various tissue types and contribute to tissue regeneration has been exploited for years and forms the basis for hundreds of clinical trials (Clinical Trials, 2008, 2017a,b).

In addition to MSCs, less versatile, tissue-specific stem cells can also be found in many adult tissues where they function to replace cellular loss due to damage or ageing. These progenitor cells can be isolated, expanded ex vivo and delivered back to the tissue of origin or to tissue locales with similar cellular makeup for restoration or supplementation of tissue function (Clinical Trials, 2007, 2013; Williams et al., 2016, 2017).

Perinatal Stem Cells

Several sources of stem cells become available in conjunction with the process of birth, including umbilical cord blood and tissue (Wharton's jelly and umbilical vein), amniotic membrane, amniotic fluid and placenta. One example of amnion cells described early on includes published work demonstrating the high plasticity and proliferative capability of the cells without the concern for tumorigenesis (De Coppi et al., 2007). These cells have since been studied for the functional use in numerous preclinical models, for example, osteogenesis imperfecta and necrotising enterocolitis that both constitute unmet clinical needs (Jones et al., 2014; Zani et al., 2014). As these tissues are usually discarded postbirth, they provide an extremely accessible and less controversial vehicle for stem cell isolation. Moreover, stem cells derived from perinatal sources display characteristics intermediate to embryonic and adult stem cells, including immunomodulatory properties, generally no tumorigenicity and high capacities for proliferation and differentiation (Abumaree et al., 2017; Zhang et al., 2017). Current clinical trials target autologous treatment of infant related diseases (Clinical Trials, 2012, 2015a) and the use of banked cells for allogeneic treatment in adults (Clinical Trials, 2015b, 2016).

BIOMATERIALS

Throughout the body, tissues and organs rely on three-dimensional (3D) architecture and ECM deposition to organise the cellular environment and maintain normal physiology (Olson et al., 2011). To recapitulate this structure–function relationship in the fields of tissue and organ regeneration, various forms of biomaterials have been utilised to provide scaffolds that aid in the retention and growth of cells. While the predominant role of a biomaterial is to provide mechanical integrity, it is also necessary that the material be inert, pose no safety issues such as toxicity or rejection and in some cases demonstrate degradation over time (Huebsch and Mooney, 2009). Additionally, scaffold design should include porosity to permit the adequate exchange of nutrients and gases and the removal of metabolic byproducts, thereby recreating an environment conducive to supporting cellular proliferation and ingrowth (Dhandayuthapani et al., 2011). As summarised in Table 9.1, biomaterials currently used in regenerative medicine include those

Table 9.1 Types of Biomaterials Used in Tissue and Organ Regeneration.

Scaffold Types	Examples
Acellular tissue matrix	Bladder tissue, heart tissue, aortic valves, small intestinal submucosa (SIS), blood vessels (aortic, carotid arteries), lung, trachea, dermal tissue, cornea, liver, adipose tissue, amniotic membrane, placental membrane, corpus cavernosum
Manufactured scaffolds – natural materials	Collagen, fibrin, fibrinogen, elastin, chitosan, chitin, keratin, dextran, alginate, gelatin, cellulose, hyaluronic acid (HA), silk fibroin, actin, myosin, amylose, glycosaminoglycans (GAG)
Manufactured scaffolds – synthetic polymers	Polyglycolic acid (PGA), polylactic acid (PLA), poly(lactic-co-glycolic) acid (PLGA), polycaprolactone (PCL), poly(caprolactone-co-ethyl ethylene phosphate) (PCLEEP), polydioxane (PDS), polyethylene glycol (PEG), polyglactin (Vicryl), polyhydroxyalkanoates (PHA), poly-N-(2-hydroxyethyl)methacrylamide (PHEMA), poly-N-(2-hydroxypropyl)methacrylamide (PHPMA)

nonimplanted materials for guiding cell growth in vitro and those implanted scaffold materials that are such as acellular tissue matrix, natural materials and synthetic polymers (Sampogna et al., 2015).

Implanted Scaffolds

Biomaterial scaffolds are used as the support structures for cells to replicate the shape of the tissue or organ to be repaired or replaced. Usually seeded with autologous cells, these constructs may be implanted as all or part of a tissue-engineered organ. As summarised in Table 9.1 and detailed below, scaffolds comprise biological or synthetic materials. The synthetic scaffolds may be produced in bulk and seeded with cells; however, 3D bioprinting offers the possibility of providing a complete organ or tissue in one step customised to the anatomy of the recipient.

Acellular Tissue Matrix

Decellularised scaffolds have been produced from the tissues of various species (Hoshiba et al., 2010). Although decellularised, these scaffolds maintain endogenous ECM composition and biomechanical properties and retain their anatomical micro- and macrostructure. Acellular tissue matrix scaffolds have the advantage of retaining a highly complex construction that currently cannot be achieved with a synthetic scaffold (Huebsch and Mooney, 2009). This complex geometry and spatial arrangement often delivers signalling cues for cells to grow and develop properly; the tissue matrix may also contain growth factors, bifunctional molecules and cryptic peptides that contribute important information regarding tissue functionality (Badylak et al., 2011). As these tissues provide the most desired anatomical shape, it makes sense that acellular allogeneic

tissues would be the natural choice as a matrix for whole-organ tissue engineering. Limited availability and high cost has, however, hindered the use of human-derived tissue-engineered products clinically (Pirnay et al., 2015). Consequently, tissues from other species including nonhuman primates, bovine, equine and porcine have been utilised as sources of acellular tissue matrices (Luo et al., 2017; Cooper, 2012). Porcine sources have tended to be favoured due to availability, organ size, low risk of xenozoonosis and fewer ethical concerns (Cooper, 2012).

Native tissues require decellularisation to become acellular tissue matrices. Decellularisation is achieved via physical, chemical or enzymatic treatments that remove cellular material and permit the retention of endogenous ECM constituents (Hoshiba et al., 2010). Physical treatments that facilitate decellularisation include cycles of freezing and thawing, direct mechanical force, sonication and freeze-drying (Kawecki et al., 2017), whereas chemical methods include the use of ionic detergents (sodium dodecyl sulphate, sodium deoxycholate), nonionic detergents (triton X-100), zwitterionic detergents (3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate) and chelating agents (ethylenediaminetetraacetic acid) (Kawecki et al., 2017). Enzymatic agents such as trypsin, dispase, nucleases and collagenases may also be used in the process (Kawecki et al., 2017). Tissues are considered decellularised if the following criteria are met: <50 ng double stranded DNA is present per mg ECM dry weight, <200 bp DNA fragment length is detected and analysis of tissue sections with 4',6-Diamidine-2'-phenylindole dihydrochloride (DAPI) or H&E staining demonstrates the absence of cells (Crapo et al., 2011).

Currently, research to evaluate host immune response to biological scaffolds derived from various mammalian ECM is underway. One xenogeneic antigen that is abundant on the glycolipids and glycoproteins of nonprimate mammals, for example, the Gal epitope (α-Gal (Galα1,3-Galβ1–4GlcNAc-R)), can elicit a response in humans. Studies have shown that this antigen can be effectively removed from tissues, however, by enzymatic digest with α-galactosidase. Going forward, tissues could potentially be derived from Gal-epitope knockout animals to avoid any hyperacute rejection clinically (Badylak and Gilbert, 2008).

Manufactured Scaffolds: Natural Materials and Synthetic Polymers

Manufactured scaffolds, whether derived from natural or synthetic materials, allow for customisation based on the needs of the application and the incorporation of distinct criteria such as chemical and mechanical properties, degradation profiles, surface structure, shape, solubility and biocompatibility (Dhandayuthapani et al., 2011). Natural materials tend to have ideal properties for cell interaction (Dang and Leong, 2006). Synthetic materials, on the other hand, tend to be more economical, uniformly produced and have better reproducibility (Dhandayuthapani et al., 2011). Scaffolds can provide more than just a structural platform for cells; receptors on the cell surface can

interact with scaffold components to drive appropriate cellular activities such as proliferation, migration and haemostasis (Sell et al., 2010). Consequently, bulk incorporation of constituents such as growth factors, proteins, peptides, and reactive sites into the scaffold may promote cell attachment and growth to develop functional tissues (Place et al., 2009). Accordingly, blends of natural and synthetic materials are often used to combine the desired properties from individual components (Sionkowska, 2011).

Manufactured scaffolds can be broadly divided into hydrogels and solid scaffolds (Knight and Przyborski, 2015). Solid scaffolds consist of cross-linked polymer chains, whereas hydrogels are semisolid and consist of cross-linked polymer chains that are highly hydrated (Place et al., 2009). Hydrogels, generated by self-assembly, ionic cross-linking or radical polymerisations by UV exposure, support the 3D culture of cells through encapsulation (Knight and Przyborski, 2015). Solid scaffolds are categorised as fibrous or porous. Fibrous materials can be created by electrospinning, wet spinning, biospinning, interfacial complexation, microfluidic spinning and melt spinning (extrusion) (Tamayol et al., 2013). The fibres generated by these processes can then be formed into scaffolds using conventional textile techniques such as weaving, knitting, felting and braiding (Tamayol et al., 2013). Porous scaffolds resemble sponge materials, with pores that can be created by particulate leaching or foaming techniques (Place et al., 2009).

Three-Dimensional Bioprinting

3D printing has emerged as a promising technology for the production of complex, precision-based scaffold structures. Multiple techniques can be utilised in printing 3D scaffolds, including direct 3D printing (layer-by-layer), fused deposition modelling, stereolithography and selective laser sintering (Do et al., 2015). Depending on the technique used, nanoscale-level precision printing can be achieved using this technique (Do et al., 2015). 3D bioprinting holds the future promise to print seeded scaffolds directly from digitised organ files, providing the polymer support structure and all required cell types and potentially an organ ready for transplant, in one application. Introduction of a complicated production device such as a bioprinters into manufacturing requires detailed instrument qualification to ensure robust operation and development of custom shielding and containment for sterility of the construct and protection of the operator.

UPCOMING TECHNOLOGIES

The basic cell therapy approach is to implant or infuse a selected cell type (usually autologous) to enhance the native regeneration and repair process for a target defect. Upcoming technologies seek to use advanced methods for higher-order repair. These include implanting functional genetic material, providing broader allogeneic treatments to meet demand, and production and improvement of more complex organ systems for tissue and organ replacement.

Genetic Modifications – Gene- and Cell-Based Therapies

A next generation approach to regenerative medicine is evolving through the use of MSCs as gene delivery vehicles to stimulate tissue regeneration. MSCs can be infused systemically for migration to areas of tissue damage. Alternatively, modification of MSCs through viral transduction (Pollock et al., 2016) or gene editing (Cho et al., 2017) can serve to deliver transgenes, the products of which can restore reparative properties or supplement endogenous functions. Technologies such as these, which are subjected to tight regulatory requirements, have reached the clinical trial phase, with some becoming licenced technologies; others are expected to reach clinical trials in the next few years. Novartis (Basel, Switzerland) and Gilead (formally Kite Pharma) (Foster City, CA, USA) have emerged as leaders in ex vivo gene therapy as the market has gained popularity, and they provided the first chimeric antigen receptor (CAR) T cell therapy FDA licenced product to be available for patients (Fernandez, 2017; Philippidis, 2017).

Allogenic (Off-the-Shelf) Treatments

Ideally, o meet both clinical and economic demands, cell-based therapies will need to be readily available, accessible and have demonstrated efficacy. One option that would facilitate these requirements is the establishment of well-characterised master and working cell banks for use in the generation of off-the-shelf therapies. This concept has stimulated studies to evaluate differences between autologous- and allogeneic-based treatments in an attempt to streamline sourcing of regenerative components. Results have been particularly promising for MSCs, likely due to their low immunogenicity and immunomodulatory properties. The safety of allogeneic MSCs has been reported in phase I clinical trials that have included the treatment of ischaemic cardiomyopathy (Hare et al., 2012), intervertebral disc repair and for phase III in Graft versus Host Disease (GVHD) (Noriega et al., 2016; Najima, 2017). There have been advances in Phase II and III clinical trials using allogeneic cell therapies for cardiac repair and cancer treatment, though some of these have not demonstrated the desired improvement over existing methods, demonstrating that there is more work to be done. However, successful demonstration of feasible allogenic treatments using cell sources other than bone marrow–derived MSCs has been reported in preclinical studies for bone-ligament grafts (Mahalingam et al., 2015), the use of amnion-derived cells for bone tissue engineering (Kim et al., 2013) and cardiac remodelling after heart failure (Castellani et al., 2013). As applications for cell therapies expand and become generalised, allogeneic products may be a viable option to meet increasing demands. The goal for an allogeneic cell source is that cryopreserved banked cells or tissues (with an option for HLA-matched materials) should be readily available.

Combinatorial Therapies

A combinatorial therapy is any treatment that uses more than one drug or active agent for complete or enhanced effect. Most tissues in the human body contain more than one

cell type, each of which performs distinct functions required for optimal tissue performance, and they are functionally integrated within the ECM. Accordingly, most tissue-engineered therapies are regulated as combination products, as defined by the US FDA, because they will contain a device (the scaffold biomaterial, see below) and biological products (one or more cell types). Case in point, hollow organs such as the vagina and bladder are composed of a smooth muscle wall with a specialised epithelium lining in the lumen. Both cellular components are integral to the organ function and need to be reproduced in engineered organs with a supporting structure to ensure adequate functionality (Atala et al., 2006; Raya-Rivera et al., 2014). Differing cell types are also responsible for creating the synergistic functionality of the heart. Cardiomyocytes, while responsible for orchestrating the contractile behaviours that result in pumping blood throughout the entire body, only make up 25% of the cardiac muscle, with other cell types (mainly fibroblasts) making up the remainder of the organ mass (Jugdutt, 2003). By mimicking this cellular composition, the addition of fibroblasts and endothelial cells to cardiomyocyte patches more closely mimics the native myocardium and results in better survival and electrophysiological functionality (Stevens et al., 2009). As cell therapies advance and address more complex systems, new strategies to generate, titrate and combine multiple cell types will be required.

Tissue-Engineered Products with Scaffolds

As shown in Table 9.2, tissue-engineered products have been produced using a variety of scaffold materials and fabrication techniques. Future developments in this arena will likely focus on enhancing cell attachment, differentiation, controlled degradation and optimised physical properties, improved bioactivity and biocompatibility.

Vascularisation of Engineered Organs

Under normal physiological conditions, the vascular system provides sufficient blood flow to perfuse every tissue and ensure metabolic demands are met for optimal cell survival and function. As the scale or thickness of an engineered tissue or organ increases, conduit structures that connect transplanted cells to the systemic circulation become key in the success of a regenerative intervention. In decellularised organs, endogenous vascular networks can be repopulated with endothelial cells to reestablish vascular functionality (Robertson et al., 2014; Shirakigawa et al., 2013). In tissues created de novo, however, this option is not available. Among strategies designed to address this point is the introduction of mixed cell populations to facilitate spontaneous vascularisation (Costa-Almeida et al., 2015; Tulloch et al., 2011). Vessel formation can also be enhanced by defining microchannels in which vascular structures will be positioned prior to cell infusion. Some of these approaches include biomaterial micropatterning (Nichol et al., 2010), casting of vascular networks using sacrificial materials (Miller et al., 2012) and bioprinting (Lee et al., 2014).

Table 9.2 Examples of Tissue-Engineered Products.

Product Name	Treatment	Description	References
Autologous adipose-derived stem cells seeded on a collagen matrix scaffold (EMA-European Union)	Cancer-related lymphedema in breast cancer	Autologous adipose-derived stem cells obtained from a stromal vascular fraction seeded on a collagen matrix scaffold	2017 (Union, 2018)
Viable chondrocytes cultured within a three-dimensional (3D) hydrogel (EMA-European Union)	Articular cartilage defects of the knee	Cultured viable chondrocytes combined with a 3D structure (a hydrogel scaffold)	2017 (Union, 2018)
Keratinocytes and mesenchymal stem cells (MSCs) seeded onto acellular amniotic matrix (EMA-European Union)	Deep and extensive burns, chronic wounds and skin donor sites	Coculture of keratinocytes and MSCs isolated from human burn eschar and debrided adipose tissue cells, seeded onto acellular amniotic matrix	2016 (Union, 2018)
Maci (Vericel Corporation, Cambridge, MA, US)	Cartilage defects of the knee	First autologous cellularised scaffold; autologous cultured chondrocytes expanded and placed onto a bioresorbable porcine-derived collagen membrane that is implanted over the area where the defective or damaged tissue was removed	2016 (Release, 2016)
Adipose tissue MSCs on acellular dermal matrix.or acellular amniotic matrix (EMA-European Union)	Burns, scars, nonhealing wounds	Human burn eschar and debrided adipose tissue MSCs seeded onto acellular dermal matrix or on acellular amniotic matrix	2016 (Union, 2018)
Tissue-engineered vagina (Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC, US)	Congenital disorder (MRKHS)	Autologous epithelial and smooth muscle cells on an intestinal submucosal segment	2014 (Raya-Rivera et al., 2014)
Tissue-engineered muscle repair construct (Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC, US)	Volumetric muscle loss injury	Rat muscle derived cells on porcine bladder acellular matrix scaffolds, moving towards human muscle derived cells for clinical applications	2014 (Corona et al., 2014)

Continued

Table 9.2 Examples of Tissue-Engineered Products.—cont'd

Product Name	Treatment	Description	References
Autologous bone marrow–derived mononuclear cells on tracheal scaffold (EMA-European Union)	Trachea reconstruction subsequent to damage or stenosis due to cancer, injury or infection	Tracheal scaffold seeded with autologous bone marrow–derived mononuclear cells	2014 (Union, 2018)
Human dermal fibroblasts on bioresorbable polyglactin mesh (EMA-European Union)	Wounds and ulcers	Human dermal fibroblasts cultured on bioresorbable polyglactin mesh	2013 (Union, 2018)
GINTUIT (Organogenesis Incorporated, US)	Mucogingival conditions in adult	Allogeneic Cultured Keratinocytes and Fibroblasts in Bovine Collagen to be used as allogeneic cellularised scaffold product indicated for topical (nonsubmerged) application to a surgically created vascular wound bed in the treatment of mucogingival conditions in adults.	2012 (Union, 2018)
Renal Assist Device (RAD, Humes, RenaMed Biologics, Inc. Lincoln, RI, US)	Acute renal failure	Allogeneic primary human renal tubule cells seeded onto hollow fibres of a standard hemofilter (Fresenius F40 polysulfone)	2012 (Humes et al., 1999; Humes et al., 2004)
3D structure of demineralised bone matrix and autologous adipose-derived and differentiated osteogenic cells (EMA-European Union)	Bone defect	Tissue-like combination of osteogenic cells and demineralised bone matrix (3Dstructure of demineralised bone matrix and autologous adipose-derived and differentiated osteogenic cells)	2012 (Union, 2018)
Autologous oral mucosa cells seeded onto a membrane (EMA-European Union)	Urethral stricture	Autologous oral mucosa cells seeded onto a biodegradable membrane	2012 (Union, 2018)
Tissue-engineered urethra (Wake Forest Institute for Regenerative Medicine, Winston–Salem, NC, US)	Urethra reconstruction	Autologous urothelial and smooth muscle cells on a collagen–polyglycolic acid (PGA) composite matrix	2011 (Raya-Rivera et al., 2011)

Allogeneic human fibroblasts on biodegradable matrix (EMA-European Union)	Dermatology	Allogeneic human fibroblasts cultured onto a biodegradable matrix	2011 (Union, 2018)
Autologous cultured chondrocytes integrated in a scaffold (EMA-European Union)	Symptomatic cartilage defects in joints, e.g., knee and ankle	Autologous cultured chondrocytes integrated in a scaffold	2010 (Union, 2018)
Autologous osteoprogenitor cells, incorporated with 3D biodegradable scaffold (EMA-European Union)	Bone defects in odontostomatology and maxillofacial surgery	Autologous osteoprogenitor cells, isolated from bone marrow and expanded in vitro, incorporated, as an integral part, with 3D biodegradable scaffold	2010 (Union, 2018)
Allogeneic corneal epithelial cell sheet in amniotic membrane scaffold (EMA-European Union)	Intended for the treatment of ocular diseases	Allogeneic cultured corneal epithelial cell sheet in amniotic membrane scaffold	2009 (Union, 2018)
DIABECELL (Living Cell Technologies, Manukau, Auckland)	Diabetes	Porcine islet cells encapsulated in alginate in combination with poly-L-ornithine or poly-L-lysine	2007 (Elliott et al., 2007)
Tissue-engineered bladder (Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC, US)	End-stage bladder disease	Autologous urothelial and smooth muscle cells on a collagen–PGA composite matrix	2006 (Atala et al., 2006)
Apligraf (Organogenesis, Inc, Canton, MA, US)	Diabetic foot ulcers	Allogeneic bilayered cultured skin consisting of an upper epidermal (formed by human keratinocytes or epidermal cells that differentiate to form epidermis) and a lower dermal layer (formed by human fibroblasts or dermal cells) in a bovine collagen matrix	2000 (Veves et al., 2001)
HepatAssist (Circe Biomedical, Lexington, MA, US)	Liver failure	Extracorporeal liver support system with cryopreserved pig liver cells on a hollow fibre membrane cartridge	1999 (Mullon and Pitkin, 1999)
Dermagraft (Organogenesis, Inc, Canton, MA, US)	Diabetic foot ulcers	Allogeneic dermal fibroblasts derived from newborn human foreskin tissue and cultured in vitro onto a bioabsorbable polyglactin (Vicryl) mesh	1996 (Harding et al., 2013; Marston et al., 2003)

MRKH, Mayer-Rokitansky-Küster-Hauser.

TRANSLATING THE BENCH RESEARCH TO CLINICAL MANUFACTURING

Translational research encompasses the application of discovery and preclinical research efforts into product development studies that adopt best manufacturing practices for clinical trials. As shown in Fig. 9.2, this process provides foundational steps that ultimately target successful product release.

Translation of a tissue-engineered product from bench to clinic includes a sequence of steps in which the product idea leading into a product design is tested and a development process is established during the laboratory research. Once laboratory research establishes proof of concept, the technology is transferred to process development. Process development is two pronged: (1) assistance of test article production for definitive studies in the preclinical animal model and (2) human process development for establishing a high-quality product while scaling up for clinical manufacturing. Once technology is scaled up and optimised, the process is transferred to cGMP manufacturing where process validation is performed for clinical product manufacturing. Once validated, in the United States, the product and process are submitted to the Food and Drug Administration (FDA) in the form of an Investigational New Drug application for approval to begin patient enrolment. With continued success, the product progresses through the phases of clinical trials towards a Biological Licence Application, with market release on approval.

PROCESS DEVELOPMENT

Process development specifically involves the optimisation of procedures necessary for product manufacturing. It includes the implementation of process controls to ensure high-quality manufacturing of products in a reproducible manner while incorporating the evaluation of product safety, biocompatibility, formulation, dose, administration route and timing for delivery and pharmacokinetics, before entering human trials.

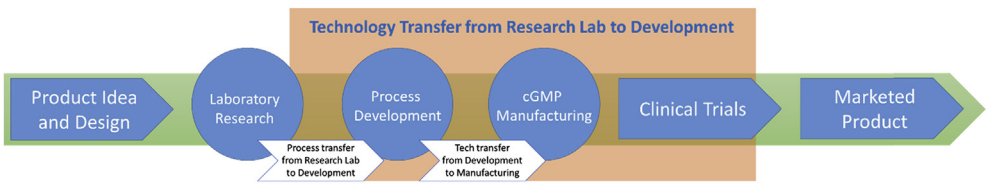

Figure 9.2 ***Steps in technology transfer from Research Lab to Development.*** Transfer of technology from research lab to clinical manufacturing occurs in stages. Once the laboratory research confirms the reliability of the technology through preclinical studies in animal models, the process is transferred to process development. On optimisation of processes and scaling up for human clinical application, Process development transfers the technology to current good manufacturing practices (cGMP) manufacturing.

Process development can be thought of in three distinct, but overlapping, phases. An early phase that includes brainstorming generalised concepts; a Middle Phase that refines target procedures; and a late phase that tests the final procedure and culminates in technology transfer to manufacturing.

Phases of Process Development

Early Phase process development activities serve as the interface with outside research efforts and begin to lay the foundation for clinical manufacturing processes. An important difference between traditional bench research and translational work is the intentional move towards compliance with regulatory requirements. While there are limited regulations and policies to be followed in the process development area, keeping an eye to the forthcoming regulatory environment in clinical manufacturing helps to ensure processes, reagents, equipment, documentation, and results are of the grade, quality and consistency required to effectively support a regulatory filing and clinical manufacturing of a product that meets the identity, potency and purity required.

The Early Phase of process development is marked with generalised, broad questions that focus on top-level areas of the development initiative such as production feasibility and patient population logistics. The focus becomes more specific in Mid-Phase development targeting process methodologies such as scaffold or material preparation, cell seeding, toxicity testing and the development of product characterisation assays. Developing assays to establish product identity, potency, purity and safety is critical to clinical translation. While some assays are standard for virtually all aseptic processes, those that target product composition and functionality will likely be unique to a specific application. Assays should be tested and qualified to ensure repeatability and accuracy. All equipment should be qualified and calibrated, and the reagents, methods and materials should be formally qualified.

During the Mid-Phase of process development, a careful review of the guidances and regulatory requirements of the agencies responsible for providing regulatory oversight should be performed. When possible, transitioning to GMP grade or pharmaceutical grade reagents and materials should be performed. Materials labelled "Research Grade Only" or "Not Intended for Clinical Use" must be vetted and qualified. If using these reagents, the user is responsible for qualifying them as safe for human use. Certifications of analysis, conformance and quality supplied from the manufacturer should be reviewed in detail to determine safety of materials components and additional testing and documentation that may be required. The process development team is integral to the collection of preclinical data and documentation that will be used in authoring submissions to health authorities during the later phase of process development.

Significant overlap generally occurs during Late-Phase process development and technology transfer into manufacturing. Similar to the generation of a medical device or a pharmaceutical, a tissue-engineered product require engineering runs, validation runs

and process qualifications to ensure that the process is robust, reproducible and limits variation. In addition, process simulations ensure operators can maintain aseptic technique throughout the manufacturing procedure. Manufacturing staff members should be involved in this phase of process development for cross-training purposes and operator qualification. While translation differs by product, several elements are a standard part of this phase of a technology transfer process including the generation of solid manufacturing batch records and Standard Operating Procedures (SOPs), verification of equipment consistency between process development and manufacturing, use of clinical grade reagents and most importantly, documentation of product safety.

Manufacturing and Scale Up

While tissue-engineered products often hold the promise of offering a cure in a single dose for numerous unmet medical needs, the complexity of the manufacturing process and the high short-term costs present unique challenges for clinical production and use of these products which will require automation as we move towards commercialisation. This section on manufacturing and scale up addresses the regulatory environment for manufacturing, cell and tissue manufacturing infrastructure, organ and tissue complexity relative to manufacturing challenges and scale up for mass production.

THE REGULATORY ENVIRONMENT

Manufacturing for cell- and tissue-based products can be defined as the sum total of the procedures, process controls, personnel, and documentation employed in generating a product for treatment of, or testing on, a human patient. Manufacturing requirements for cell- and tissue-based products are legislated by a regulatory framework defining current Good Tissue Practices (cGTPs) and cGMPs to ensure a safe product that meets its producers' claims of identity, quality, purity and potency.

In the United States, cGMPs are defined by the US FDA, which often point to standards or procedures developed by other government and nongovernment agencies such as United States Pharmacopeia, International Organization for Standardization (ISO), the Public Health Service (PHS), and the National Institute of Standards and Technology.

The following list provides a brief summary of some of the key FDA Codes of Federal Regulation (CFRs) and PHS Acts governing cGMP/cGTPs and human research, as well as nonbinding guidance documents issued for assistance or clarification in interpreting the statutes. The nature of the product defines which CFRs apply.

Representative US regulations governing cGMPs, GTPs or related tissue processing requirements:

- 9 CFR 113.53: Requirements for ingredients of animal origin used for production of biologics
- 21 CFR 4: cGMP requirements applicable to combination products (and see 21 CFR 3)

- 21 CFR 11: Electronic records; electronic signatures
- 21 CFR 210: cGMP in manufacturing, processing, packing, or holding of drugs; general
- 21 CFR 211: cGMP for finished pharmaceuticals
- 21 CFR Parts 600 through 680: Other applicable regulations for biological products
- 21 CFR 820: Quality system regulation
- 21 CFR Part 1271: Human cell, tissue and cellular and tissue-based products (HCT/Ps)
- Section 351 of the PHS Act (42 U.S.C. 262): Drugs, devices and/or biological products requiring clinical trials
- Section 361 of the PHS Act (42 U.S.C. 264): Human cells, tissues and cellular- and tissue-based products (HCT/Ps) meeting certain minimally manipulated and homologous use criteria

Representative nonbinding guidance documents provided by the FDA

- Guidance for Industry and FDA Staff: Regulatory Considerations for Human Cells, Tissues and Cellular and Tissue-Based Products: Minimal Manipulation and Homologous Use. Issued November 2017; Updated December 2017
- Guidance for Industry: Same Surgical Procedure Exception under 21 CFR 1271.15(b): Questions and Answers Regarding the Scope of the Exception, November 2017
- Draft Guidance for Industry: Evaluation of Devices Used with Regenerative Medicine Advanced Therapies. November 2017
- Draft Guidance for Industry: Expedited Programs for Regenerative Medicine Therapies for Serious Conditions. November 2017
- Draft Guidance for Industry and FDA Staff: Technical Considerations for Additive Manufactured Devices. May 10, 2016
- Draft Guidance for Industry and FDA Staff: Medical Devices Containing Materials Derived from Animal Sources (Except for In Vitro Diagnostic Devices), January 2014
- Guidance for Industry: Preclinical Assessment of Investigational Cellular and Gene Therapy Products, November 2013
- Guidance for Industry: Preparation of IDEs and INDs for Products Intended to Repair or Replace Knee Cartilage, December 2012
- Guidance for Industry: cGTP and Additional Requirements for Manufacturers of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps), December 2011
- Guidance for Industry: CGMP for Phase 1 Investigational Drugs, July 2008
- Guidance for Industry: Regulation of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) Small Entity Compliance Guide, August 2007
- Guidance for Industry: Computerised Systems Used in Clinical Investigations, May 2007

- Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing – cGMP, September 2004
- Guidance for Industry: Part 11, Electronic Records; Electronic Signatures - Scope and Application, August 2003

Cell and Tissue Manufacturing Infrastructure for cGMP Compliance

Manufacturing steps can be either open or closed. All open manufacturing steps, such as standard cell culture, must be performed in a controlled environment such as a clean room that meets certain viable and nonviable air particulate and other standards. Closed systems require that all air pass through sterilising filtres on the tissue culture devices and bioreactors, and all manipulations occur without exposing the product to the surrounding environment. This is usually accomplished using fluid bags and tubing welders, for example, for media changes and the like. The majority of early phase manufacturing occurs with at least some steps performed as open systems, for example, the tissue digestion.

Clean rooms are facilities that are engineered to provide clean air, clean surfaces, process control and personnel control. Fan-powered HEPA filters supply highly purified air to the workspace. The rate of air replacement and level of positive pressure relative to adjacent rooms help define the clean room classification based on particle count, bioburde, and intended use. The current standard in the United State is the ISO classification system partially presented in Table 9.3 with general correlation to other systems and some representative usages of the classified spaces.

For cell and tissue manufacturing, all open processing is performed in an ISO 5 biological safety cabinet (BSC, or sterile hood) placed within a higher classed space. Custom ISO 5 enclosures may be constructed around critical equipment, such as cell sorters or bioprinters. Usually, lower ISO class (cleaner) areas are positive pressure relative to adjacent areas. Operator gowning is necessary and usually designed around maintaining sterility of the product first and protection of the operator from biohazards or chemical hazards second. Notably, all materials in the clean room must be nonshedding and cleanable. In general, only the amount of new consumable supplies and reagents necessary for a single process are brought into the clean room, with excess materials being discarded or moved out to research areas to reduce particulate introduction and the risk of cross-contamination. All clean room surfaces are regularly cleaned with phenolic disinfectants and sporicidal agents, and regular environmental monitoring ensures conformance to ISO classification.

Nearly all manufacturing processes require a minimum of two operators. A primary operator performs all aseptic process steps, whereas a secondary operator provides material support and verifies performance on documentation in the form of manufacturing batch records issued by the Quality Assurance team. All manufacturing documentation is reviewed for completeness, correctness and quality by a technical supervisor and a quality assurance supervisor. Any use of electronically generated, stored, or signed data must be complian with 21 CFR 11.

Table 9.3 Clean Room Environmental Classification

ISO Class[a]	# Particles ≥0.5 µm/m³	# Particles ≥5 µm/m³	Former Fed. Standard 209E (Pre-2002) (# Particles ≥0.5 µm/ft³)[a]	EU Grade (approx.)[a]	Usage[b]
8	3,520,000	29,300	100,000	C/D	Initial processing of nonsterile tissues; process development/early translational testing
5	352,000	2930	10,000	B/C	Sterile cell manufacturing (operator space, closed samples)
6	35,200	293	1000		Sterile cell manufacturing (operator space, closed samples)
5	3,520	29	100	A/B	Sterile hood (BSC) for aseptic product formulation (open samples)

BSC, Biological Safety Cabinet; *EU*, European Union; *ISO*, International Organization for Standardization.
[a]Comparisons are highly generalised – many variations exist between systems, and many additional requirements may apply including bioburden, air exchange rates, rest v. active levels, etc.; See: ISO 14,644-1/-4, EU GGMP, and others.
[b]Usage may vary according to product-related needs and regulatory compliance requirements.

Cell Therapy Manufacturing and Product Complexity

The following list addresses manufacturing requirements or challenges for each product type by increasing level of complexity:

1. *External cell or ECM applications:* These may include allograft cell, plasma or other tissue-based gels, suspensions or tissue sheets, such as products to cover skin wounds such as burns or ulcers. These processes generally require only standard cell culture or tissue isolation and lyophilisation or cryopreservation. Application can be as simple as applying a bandage or an ointment. However, some research has been conducted on custom 3D bioprinting to exactly match the contour and depth of the wound, which would introduce a high level of complexity to the category of external application.
2. *Cell suspension infusions for internal application:* These may also be prepared by simple cell expansion either in open culture or closed systems. However, some rare cell types may require isolation using specialised cell sorting devices, thus requiring a higher level of complexity for cGMP compliance. Usually the final product is a fairly simple cell suspension in a syringe or other specialised infusion device. Instructions to the

clinical team may require that cells be agitated in the syringe before application to ensure the settled cells are administered evenly.

3. *Flat organs:* These may include skin, muscle or some articular cartilage. As an organ structure and not just a cell sheet, flat organs usually involve more complex manufacturing than just growing cells in a dish. Organs usually require more than one cell type to comprise the various tissue layers. Furthermore, flat organ structures require some depth provided by a scaffold biomaterial (see biomaterials section).
4. *Tubular hollow organs:* Cylindrical structures such as tissue-engineered urethra require specialised bioreactors for the culture environment and custom biomaterial scaffolds to provide the shape and support for the engineered organ. These processes usually require two-dimensional culture until a target cell count is achieved (usually of two or more cell types), as well as preparation and sterilisation of the scaffold and bioreactor. Cell maturation on the scaffold may take place in multiple phases depending on the number and type of cells required. Again, broader regulatory oversight applies in these combination products.
5. *Hollow spherical organs:* The process and material nature of the hollow spherical organs such as tissue-engineered bladder are similar to the tubular hollow organs. The increase in complexity is derived from the size and function of the tubular organs. The spherical organs are usually larger, have much thicker walls and have higher function, requiring advanced signal processing to develop within the organ following implantation and a higher need for deep vascularisation. Thus, nutrient exchange and respiration present a greater challenge for manufacturing.
6. *Solid organs:* These are the holy grail of tissue engineering. The structural and functional variety and complexity of solid organs such as liver or kidney makes each one a special case and can make the challenge of manufacturing these organs many orders of magnitude more difficult than the prior types. Nevertheless, the foundational methods that will make manufacturing these organs possible are being developed today. A prime example includes clinical trial stage tissue-engineered penile reconstruction using an acellular allograft scaffold to repair damaged corpus cavernosum (Chen et al., 2010; Clinical Trial, 2018). Others are still early in development such as producing a bioartificial endocrine pancreas from acellular ECM scaffolds from the human pancreas (Peloso et al., 2016).

SCALE UP

Scale up for manufacturing is defined as the process of retooling to move from small batch production to large batch production. Tissue-engineered products face a particular challenge for scale up. Most tissue-engineered products fall into the category of personalised medicine: one product is custom-produced for one patient. Consequently, the product can take weeks or months to produce, require hundreds of work hours and necessitate the engagement of customised and often costly logistical operators to reach the patient.

Scale up to meet demand and to make tissue-engineered treatments safe, less labour intensive and more cost-effective will require identifying processes that can support production for multiple patients at once. Scale up will require automation of as many parts of the process as possible. Currently, several commercially available systems provide partially automated cell isolation and expansion through the use of robotics, carrier beads or capillary tubes, such as the Quantum Cell Expansion System (Terumo BCT) and the RoboSep (STEMCELL Technologies). As scale up processes evolve, engineering controls will be required to close down systems for better containment and the elimination of operator-entry clean rooms and controlled environments; current solutions include simple, connectable enclosures such as the Series 500 glove box isolators (Terra Universal) or complex integrated automation devices such as the Cocoon (Octane). Ideally, as commercial-scale production advances, linking of 'biopsy in/final product out' processes with minimal manual intervention between transition points such as cell expansion, scaffold preparation, scaffold seeding and construct maturation will be optimal, taking the form of interconnected controlled-environment modules operated through robotics and containing parallel systems to produce for more than one patient at a time. As production increases from tens of patients in early trials to hundreds or thousands per year in a commercial environment, devising manufacturing facilities with automated assembly, digital interfaces and limited manual intervention, that reside closer to treatment locations will be paramount for successful scale up. A potential benefit of scaling towards mass production is economy of scale, resulting from redundancies in the processes or materials necessary for generating various organs. As an example, a bioreactor that can be used for multiple organ types of similar size and shape; a single automated processing facility would only require process reprogramming and reagent substitutions to make different organs.

The question is when and for which applications could or should scale up be implemented? The list of applications with needs for this type of intervention is in the tens to hundreds of thousands per year, a point for consideration despite the high initial input costs and complexities of generating these type therapies. Equally important, however, is that the return on investment extends far past monetary gain to the potential to save lives and reduce the prohibitive healthcare costs that accrue from decades of supportive medical care.

PERSPECTIVES: REGENERATIVE MEDICINE CLINICAL TRANSLATION, A CHANGING PARADIGM

Regenerative medicine applications are now rapidly changing the way healthcare is delivered today with the recent approval of several Cell and Gene Therapy Products in the United States and abroad. At the same time, we are seeing the maturation of tissue-engineered organs making their way to the clinic. One of the first solid organ structures (bioengineered corporal tissue for penile reconstruction) has been approved by FDA for

Phase 1 clinical trial study. Several 3D constructs with chondrocytes have been approved for clinical trials or market approval to manufacture cartilage. Several flat structures such as skin and hollow organs such as tissue-engineered bladder, urethra, vagina and trachea have been in clinical trials or exploratory studies. We are watching for the next dimension of tissue-engineered constructs being manufactured via 3D bioprinting, certainly an exciting time in regenerative medicine. Key hurdles in the field of tissue engineering remain the same as many are attempting to optimise vascularity in tissue-engineered organs/structures and recapitulate the intense architecture of solid organs using many different methods for scaffolding from synthetic to natural biomaterials. There are key hurdles in 3D bioprinting that allow the methodology to follow other manufacturing processes for tissue-engineered structures including imaging, software, availability of GMP bioprinters and biomaterials that can be used as a universal bioink. Hopefully within the next 5 years we will see 3D bioprinting being used for manufacturing of tissues or structures for implant.

In the United States particularly, one is witnessing the results of the US FDA efforts including several accelerated pathways. Notably, the year 2017 brought the 21st Century Cures Act into Law that not only facilitates an additional accelerated pathway specifically for Regenerative Medicine but also covers significant ground regarding a patient-focused drug development. FDA is required to develop guidance documents that include patient experience data and the use of the data in drug development (Guidance, 2017). This change will allow patients a voice in their healthcare and enhance transparency on impact of the therapy. For the first time in history, gene therapy products are being approved. One of the main challenges to drug development is the overall cost. In 2016, the Tufts Center for the Study of Drug Development published a new assessment of the major cost to develop and gain licensure of a new drug; these findings were published in the Journal of Healthcare Economics with an estimate of $2.558 billion and with out-of-pocket costs at about $1.4 billion (DiMasi et al., 2016). We are looking forward to the impact that regulatory acceleration will have on overall drug development costs. A key trend already emerges in manufacturing where automation is increasingly implemented at various stages such as at the cell expansion step. The future will bring full automation to a process where possibly decisions can be made by collection of big data (including genomics and other omics, patient clinical data, product characterisation and imaging to list only a few) and artificial intelligence. The field of tissue engineering incorporating scaffolds and cells will require significantly higher levels of deeply integrated automation for processing to control cost of goods prior to commercialisation of these combination products; one the other hand, this new capability to manufacture functional humanised organs de novo has the potential to change healthcare economics forever. We are now seeing more investor confidence as regenerative medicine is moving to more mature stages of clinical trials. Over the past 5 years a significant number of products in Phase 1 clinical trials moved to Phase 2 clinical trials with more than 50% currently in phase 2

and a significant number of trials in Phase 3. Clinical translation of regenerative medicine and tissue engineering has nowadays a significant momentum that will certainly accelerate over the next year with regulatory considerations in place and all sectors of the healthcare field convening at meetings working as a team to move the innovative science to the bedside for unmet clinical needs.

REFERENCES

Abumaree MH, et al. Immunomodulatory properties of human placental mesenchymal stem/stromal cells. Placenta 2017;59:87–95.

Atala A, et al. Tissue-engineered autologous bladders for patients needing cystoplasty. Lancet 2006;367(9518):1241–6.

Badylak SF, Gilbert TW. Immune response to biologic scaffold materials. Semin Immunol 2008;20(2):109–16.

Badylak SF, Nerem RM. Progress in tissue engineering and regenerative medicine. Proc Natl Acad Sci USA 2010;107(8):3285–6.

Badylak SF, Taylor D, Uygun K. Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix scaffolds. Annu Rev Biomed Eng 2011;13:27–53.

Bell E, Ivarsson B, Merrill C. Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. Proc Natl Acad Sci USA 1979;76(3):1274–8.

Cao Y, et al. Transplantation of chondrocytes utilizing a polymer-cell construct to produce tissue-engineered cartilage in the shape of a human ear. Plast Reconstr Surg 1997;100(2):297–302. Discussion 303–4.

Castellani C, et al. The contribution of stem cell therapy to skeletal muscle remodeling in heart failure. Int J Cardiol 2013;168(3):2014–21.

Chen KL, et al. Bioengineered corporal tissue for structural and functional restoration of the penis. Proc Natl Acad Sci USA 2010;107(8):3346–50.

Cho HM, et al. Targeted genome engineering to control VEGF expression in human umbilical cord blood-derived mesenchymal stem cells: potential implications for the treatment of myocardial infarction. Stem Cells Transl Med 2017;6(3):1040–51.

Clinical Trial. Bioengineered penile tissue constructs for irreversibly damaged penile corpora. 2018.

Clinical Trials. Cardiac stem cell infusion in patients with ischemic cardiomyopathy (SCIPIO). National Library of Medicine (US): Bethesda (MD); 2007.

Clinical Trials. The transendocardial autologous cells (hMSC or hBMC) in ischemic heart failure trial (TAC-HFT). National Library of Medicine (US): Bethesda (MD); 2008.

Clinical Trials. Autologous cord blood stem cells for autism. National Library of Medicine (US): Bethesda (MD); 2012.

Clinical Trials. Muscle progenitor cell therapy for urinary incontinence. National Library of Medicine (US): Bethesda (MD); 2013.

Clinical Trials. Transplantation of human embryonic stem cell-derived progenitors in severe heart failure. National Library of Medicine (US): Bethesda (MD); 2014.

Clinical Trials. A multi-site study of autologous cord blood cells for hypoxic ischemic encephalopathy. National Library of Medicine (US): Bethesda (MD); 2015.

Clinical Trials. Cord blood infusion for ischemic stroke. National Library of Medicine (US): Bethesda (MD); 2015.

Clinical Trials. Transplantation of ex vivo expanded, UCB-derived, stem & progenitor cells vs. unmanipulated UCB for HM patients. National Library of Medicine (US): Bethesda (MD); 2016.

Clinical Trials. Phase I mesenchymal stem cells for systemic lupus erythematosus. National Library of Medicine (US): Bethesda (MD); 2017.

Clinical Trials. Intrathecal administration of autologous mesenchymal stem cell-derived neural progenitors (MSC-NP) in progressive multiple sclerosis. Transplantation of Human Embryonic Stem Cell-derived Progenitors in Severe Heart Failure. National Library of Medicine (US): Bethesda (MD); 2017.

Colombo D, Ammirati E. Cyclosporine in transplantation - a history of converging timelines. J Biol Regul Homeost Agents 2011;25(4):493–504.
Cooper DK. A brief history of cross-species organ transplantation. Proc (Bayl Univ Med Cent) 2012;25(1):49–57.
Corona BT, et al. Implantation of in vitro tissue engineered muscle repair constructs and bladder acellular matrices partially restore in vivo skeletal muscle function in a rat model of volumetric muscle loss injury. Tissue Eng Part A 2014;20(3–4):705–15.
Costa-Almeida R, et al. Fibroblast-endothelial partners for vascularization strategies in tissue engineering. Tissue Eng Part A 2015;21(5–6):1055–65.
Crapo PM, Gilbert TW, Badylak SF. An overview of tissue and whole organ decellularization processes. Biomaterials 2011;32(12):3233–43.
Dang JM, Leong KW. Natural polymers for gene delivery and tissue engineering. Adv Drug Deliv Rev 2006;58(4):487–99.
De Coppi P, et al. Isolation of amniotic stem cell lines with potential for therapy. Nat Biotechnol 2007;25(1):100–6.
Dhandayuthapani B, et al. Polymeric scaffolds in tissue engineering application: a review. Int J Polym Sci 2011:2011.
DiMasi JA, Grabowski HG, Hansen RW. Innovation in the pharmaceutical industry: new estimates of R&D costs. J Health Econ 2016;47:20–33.
Do AV, et al. 3D printing of scaffolds for tissue regeneration applications. Adv Healthc Mater 2015;4(12):1742–62.
Elliott RB, et al. Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation. Xenotransplantation 2007;14(2):157–61.
Fernandez CR. The FDA approves a second CAR-T therapy cheaper than Novartis. Labiotech.eu; October 19, 2017. [News Article]. Available from: https://labiotech.eu/yescarta-kymriah-car-t-therapy/.
Harding K, Sumner M, Cardinal M. A prospective, multicentre, randomised controlled study of human fibroblast-derived dermal substitute (Dermagraft) in patients with venous leg ulcers. Int Wound J 2013;10(2):132–7.
Hare JM, et al. Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial. JAMA 2012;308(22):2369–79.
Hoshiba T, et al. Decellularized matrices for tissue engineering. Expert Opin Biol Ther 2010;10(12):1717–28.
Huebsch N, Mooney DJ. Inspiration and application in the evolution of biomaterials. Nature 2009;462(7272):426–32.
Humes HD, et al. Tissue engineering of a bioartificial renal tubule assist device: in vitro transport and metabolic characteristics. Kidney Int 1999;55(6):2502–14.
Humes HD, et al. Initial clinical results of the bioartificial kidney containing human cells in ICU patients with acute renal failure. Kidney Int 2004;66(4):1578–88.
Jones GN, et al. Potential of human fetal chorionic stem cells for the treatment of osteogenesis imperfecta. Stem Cells Dev 2014;23(3):262–76.
Jugdutt BI. Ventricular remodeling after infarction and the extracellular collagen matrix: when is enough enough? Circulation 2003;108(11):1395–403.
Kawecki M, et al. A review of decellurization methods caused by an urgent need for quality control of cell-free extracellular matrix' scaffolds and their role in regenerative medicine. J Biomed Mater Res B Appl Biomater 2017. n/a–n/a.
Kim J, et al. In vitro osteogenic differentiation of human amniotic fluid-derived stem cells on a poly(lactide-co-glycolide) (PLGA)-bladder submucosa matrix (BSM) composite scaffold for bone tissue engineering. Biomed Mater 2013;8(1):014107.
Knight E, Przyborski S. Advances in 3D cell culture technologies enabling tissue-like structures to be created in vitro. J Anat 2015;227(6):746–56.
Lee VK, et al. Generation of multi-scale vascular network system within 3D hydrogel using 3D bio-printing technology. Cell Mol Bioeng 2014;7(3):460–72.

Luo X, et al. In vitro evaluation of decellularized ECM-derived surgical scaffold biomaterials. J Biomed Mater Res B Appl Biomater 2017;105(3):585–93.
Macchiarini P, et al. Clinical transplantation of a tissue-engineered airway. Lancet 2008;372(9655):2023–30.
Mahalingam VD, et al. Allogeneic versus autologous derived cell sources for use in engineered bone-ligament-bone grafts in sheep anterior cruciate ligament repair. Tissue Eng Part A 2015;21(5–6):1047–54.
Marston WA, et al. The efficacy and safety of Dermagraft in improving the healing of chronic diabetic foot ulcers: results of a prospective randomized trial. Diabetes Care 2003;26(6):1701–5.
Michalopoulos GK. Liver regeneration after partial hepatectomy: critical analysis of mechanistic dilemmas. Am J Pathol 2010;176(1):2–13.
Miller JS, et al. Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. Nat Mater 2012;11(9):768–74.
Mullon C, Pitkin Z. The HepatAssist bioartificial liver support system: clinical study and pig hepatocyte process. Expert Opin Investig Drugs 1999;8(3):229–35.
Murray JE. The first successful organ transplants in man. J Am Coll Surg 2005;200(1):5–9.
Najima Y. [Mesenchymal stem cells for treatment of graft-versus-host disease]. Rinsho Ketsueki 2017;58(12):2440–9.
Nichol JW, et al. Cell-laden microengineered gelatin methacrylate hydrogels. Biomaterials 2010;31(21):5536–44.
Noriega DC, et al. Intervertebral disc repair by allogeneic mesenchymal bone marrow cells: a randomized controlled trial. Transplantation 2017;101(8):1945–71551.
O'Connor NE, Mulliken J, Banks-Schlegel S, Kehinde O, Green H. Grafting of burns with cultured epithelium prepared from autologous epidermal cells. Lancet 1981;1(8211):75–8.
Olson JL, Atala A, Yoo JJ. Tissue engineering: current strategies and future directions. Chonnam Med J 2011;47(1):1–13.
Peloso A, et al. The human pancreas as a source of protolerogenic extracellular matrix scaffold for a new-generation bioartificial endocrine pancreas. Ann Surg 2016;264(1):169–79.
Philippidis A. FDA panel unanimously recommends approval for Novartis' CAR T-cell therapy CTL019. Genet Eng Biotechnol News July 13, 2017. [News Article]. Available from: www.genengnews.com/gen-news-highlights/fda-panel-unanimously-recommends-approval-for-novartis-car-t-cell-therapy-ctl019/81254648.
Pirnay J-P, et al. Access to human tissues for research and product development: from EU regulation to alarming legal developments in Belgium. EMBO Rep 2015;16(5):557–62.
Place ES, et al. Synthetic polymer scaffolds for tissue engineering. Chem Soc Rev 2009;38(4):1139–51.
Pollock K, et al. Human mesenchymal stem cells genetically engineered to overexpress brain-derived neurotrophic factor improve outcomes in huntington's disease mouse models. Mol Ther 2016;24(5):965–77.
Raya-Rivera A, et al. Tissue-engineered autologous urethras for patients who need reconstruction: an observational study. Lancet 2011;377(9772):1175–82.
Raya-Rivera AM, et al. Tissue-engineered autologous vaginal organs in patients: a pilot cohort study. Lancet 2014;384(9940):329–36.
Release FP. FDA approves first autologous cellularized scaffold for the repair of cartilage defects of the knee. 2016.
Robertson MJ, et al. Optimizing recellularization of whole decellularized heart extracellular matrix. PLoS One 2014;9(2):e90406.
Ruijtenberg S, van den Heuvel S. Coordinating cell proliferation and differentiation: antagonism between cell cycle regulators and cell type-specific gene expression. Cell Cycle 2016;15(2):196–212.
Sampogna G, Guraya SY, Forgione A. Regenerative medicine: historical roots and potential strategies in modern medicine. J Microsc Ultrastruct 2015;3(3):101–7.
Sell SA, et al. The use of natural polymers in tissue engineering: a focus on electrospun extracellular matrix analogues. Polymers 2010;2(4).
Shirakigawa N, Takei T, Ijima H. Base structure consisting of an endothelialized vascular-tree network and hepatocytes for whole liver engineering. J Biosci Bioeng 2013;116(6):740–5.

Sionkowska A. Current research on the blends of natural and synthetic polymers as new biomaterials: review. Prog Polym Sci 2011;36(9):1254–76.
Stevens KR, et al. Physiological function and transplantation of scaffold-free and vascularized human cardiac muscle tissue. Proc Natl Acad Sci USA 2009;106(39):16568–73.
Takahashi K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131(5):861–72.
Tamayol A, et al. Fiber-based tissue engineering: progress, challenges, and opportunities. Biotechnol Adv 2013;31(5):669–87.
Thomas ED, et al. Supralethal whole body irradiation and isologous marrow transplantation in man. J Clin Investig 1959;38:1709–16.
Trounson A, DeWitt ND. Pluripotent stem cells progressing to the clinic. Nat Rev Mol Cell Biol 2016;17(3):194–200.
Tulloch NL, et al. Growth of engineered human myocardium with mechanical loading and vascular coculture. Circ Res 2011;109(1):47–59.
Union E-E. Summaries of scientific recommendations on classification of advanced therapy medicinal products. 2018. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000301.jsp&murl=menus/regulations/regulations.jsp&mid=WC0b01ac05800862c0.
US Food and Drug Administration/Center for Drug Evaluation and Research. FDA plan for issuance of patient-focused drug development guidance. FDA public health advisory. Washington, DC 2017.
Vacanti JP, et al. Selective cell transplantation using bioabsorbable artificial polymers as matrices. J Pediatr Surg 1988;23(1 Pt 2):3–9.
Vacanti JP. Beyond transplantation. Third annual Samuel Jason Mixter lecture. Arch Surg 1988;123(5):545–9.
Vacanti J. Tissue engineering and regenerative medicine: from first principles to state of the art. J Pediatr Surg 2010;45(2):291–4.
Veves A, et al. Graftskin, a human skin equivalent, is effective in the management of noninfected neuropathic diabetic foot ulcers: a prospective randomized multicenter clinical trial. Diabetes Care 2001;24(2):290–5.
Williams JK, et al. Local versus intravenous injections of skeletal muscle precursor cells in nonhuman primates with acute or chronic intrinsic urinary sphincter deficiency. Stem Cell Res Ther 2016;7(1):147.
Williams JK, et al. Determinates of muscle precursor cell therapy efficacy in a nonhuman primate model of intrinsic urinary sphincter deficiency. Stem Cell Res Ther 2017;8(1):1.
Wolter JR, Meyer RF. Sessile macrophages forming clear endotheliumlike membrane on the inside of successful keratoprosthesis. Graefes Arch Clin Exp Ophthalmol 1985;222(3):109–17.
Zani A, et al. Amniotic fluid stem cells improve survival and enhance repair of damaged intestine in necrotising enterocolitis via a COX-2 dependent mechanism. Gut 2014;63(2):300–9.
Zhang H, et al. Immunomodulatory function of whole human umbilical cord derived mesenchymal stem cells. Mol Immunol 2017;87:293–9.