REVIEW

National Science Review
3: 331–344, 2016
doi: 10.1093/nsr/nww037
Advance access publication 7 June 2016

BIOLOGY & BIOCHEMISTRY

Three-dimensional bioprinting speeds up smart regenerative medicine

Qi Gu[1,2,†], He Zhu[1,†], Jing Li[1], Xia Li[1], Jie Hao[1], Gordon G. Wallace[2] and Qi Zhou[1,*]

ABSTRACT

Biological materials can actively participate in the formation of bioactive organs and can even control cell fate to form functional tissues that we name as the smart regenerative medicine (SRM). The SRM requires interdisciplinary efforts to finalize the pre-designed organs. Three-dimensional (3D) printing, as an additive manufacturing technology, has been widely used in various fields due to its high resolution and individuation. In SRM, with the assistance of 3D printing, cells and biomaterials could be precisely positioned to construct complicated tissues. This review summarizes the state of the SRM advances and focuses in particular on the 3D printing application in biofabrication. We further discuss the issues of SRM development and finally propose some approaches for future 3D printing, which involves SRM.

Keywords: 3D bioprinting, regenerative medicine, biomaterials, tissues

[1]State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China and [2]ARC Centre of Excellence for Electromaterials Science (ACES), Intelligent Polymer Research Institute, AIIM Facility, Innovation Campus, University of Wollongong, NSW 2522, Australia

[†]Equally contributed to this work.

[*]**Corresponding author.** E-mail: qzhou@ioz.ac.cn

Received 7 December 2015; **Revised** 13 May 2016; **Accepted** 16 May 2016

INTRODUCTION

The rapid growth of organ-transplantation demand is outnumbering the donors. Although 1 million people benefit from organ transplantation globally, the death toll of patients while on the transplant waiting list is up to 15%–30% [1]. Regenerative medicine, a broad field including tissue engineering and cell therapy, is a promising approach to restore damaged tissues and their normal function by regenerating cells or tissues with the help of external factors such as physical and chemical conditions including pressure, nutrients, electrical signals and communication with other cells [2]. Since the first approval of a cell-based therapy product for treating serious burns [3] by Food and Drug Administration (FDA), many significant advances in this field have resulted in achieving its potential in improving the lives of countless patients. Regenerative medicine or tissue engineering works by delivering the cells and material constructs fabricated *in vitro* into the body as therapeutic assistance aiming to restore original function with or without low transplant rejection by the host.

Biomaterials, typically multifunctional, have been used as part of medical devices such as artificial bones [4], artificial heart valve or joint [5], artificial nerve conduit [6], artificial cochlea [7] and even artificial eyes [8], and it is reported that the entire artificial heart is under animal model tests [9]. Biomaterials can be divided into three categories: synthetic, naturally derived and hybrid materials [10]. Over the last few years, there is a transition from the use of synthetic materials such as metals and ceramics to natural derivatives in clinical medicine, which consist of mechanical and biological properties more similar to the native tissues. Therefore, combining cells with biomaterials provide a promising strategy in regenerative medicine because healthy cells introduced into the damaged tissues could help to replace or restore the tissue [11]. Some biomaterials could be used as scaffolds to improve the biofunction, or as delivery carriers to locate the cells in cell therapy such as an FDA-approved product OrCel, which is a bilayered cellular matrix that cultures dermal fibroblasts as skin substitutes [12].

Cells are the basic unit of organisms. Each organ is composed of various cell types and the function is dependent on the multicellular interaction. Three-dimensional (3D) printing holds potential to reproduce complex bionic devices or fabricate organs with intricate 3D micro-architecture *in vitro*. 3D printing,

also known as additive manufacturing and rapid prototyping, was initially described as stereolithography [13]. It is a methodology using 3D computer-aided design (CAD) data to produce 3D structure layer by layer. The main features of 3D printing are its specialization and high resolution. It could complete the structure as the imagination, faster and cheaper. Currently, 3D printing in biological application has been reflected in creating clinical devices for implantation, patterning arrays in the drug screening system, cell encapsulation via direct writing and even reconstructing an entire organ [14]. Therefore, 3D bioprinting is a highly promising tool in regenerative medicine.

In recent years, various approaches have been explored in regenerative medicine. The generated tissues account for macro, micro, nanostructure, nutrients and wastes removal transport and cell-matrix, cell-biomolecules communication for cellular adhesion, proliferation and function [15]. Ideally, with the development of materials and manufacturing techniques such as 3D bioprinting, a type of smart regenerative medicine (SRM) is outlined as below: an organ structure is reconstructed by 3D bioprinting seed cells and matrix materials, and after some self-reorganization, it will be a true organ with normal function.

Here, we review the trend of regenerative medicine, propose and define SRM. This review focuses on the present application of 3D bioprinting in tissue engineering with emphasis on organ and bionic organ construction. We also review the present material-involved clinical products and envisage 3D bioprinting application in SRM.

REGENERATIVE MEDICINE

Regenerative medicine, a term invented in 1999 by William Haseltine, is an emerging interdisciplinary field of research and clinical applications [16], which focuses on the process of replacing, engineering or regenerating human cells, tissues or organs to restore or establish normal functions that are similar to original ones [17] via means of cell/stem cell transplantation, tissue engineering, nuclear transfer or materials science [18]. Regarding that, stem cells have a great potential in regenerative medicine due to their self-renewing and differentiating capability. The methods of stem-cell-based therapy contain injection of stem-cells-derived specialized cells into damaged sites, infusion of biologically active molecules secreted by stem cells for regeneration induction, possible growth of tissues or organs *in vitro* for transplantations into patients whose damaged organs are no longer able to self-repair [19,20]. In addition to using the patient's own tissue or cells to regenerate organ, the regenerated organ could potentially solve the problem of organ shortage and transplant rejection [17,21].

Regenerative medicine strategies generally fall into two categories: cell-based and non-cell-based regenerative medicine [22]. For non-cell-based regenerative medicine, synthetic materials and biomaterials are utilized to replace diseased tissues or parts in the human body. The advent of man-made materials such as Teflon [23] and silicone has created new prospects such as a wide array of devices that can be used in humans. Man-made tissues existing already in clinical use include artificial bones [4], artificial heart valve or joint [5], artificial nerve conduit [6], artificial cochlea [7] and so on. However, although these devices provided structural replacement, the function of the original tissue was still not restored to normal. As for cell-based regenerative medicine, cells can be delivered by direct injection or using an appropriate biomaterial structures such as hydrogels as carriers [24]. Biomaterials provide a 3D environment for cell survival, attachment and new tissue formation of appropriate structure and function [25]. Single cell type could not meet the requirements for the organ replacement or the reconstruction of parts of organs. With the development of tissue engineering and additive technologies, it is possible to fabricate organs *in vitro* by perfect multidisciplinary research. So, the regenerative medicine has upgraded to SRM now. In SRM, biomaterials act as a pivotal medium to communicate with cells and circumcentres in the body [26]. Here, we will describe the specific biomaterials and stem cells that involved regenerative medicine.

Clinical application of stem cells

Now, there has been a growing interest in stem cells, including pluripotent stem cells and adult stem-cells-based clinical treatment (https://clinicaltrials.gov/). Embryonic stem cells (ESCs) and induced pluripotent stem cells are pluripotent stem cells that can differentiate into any types of cells of the three embryonic germ layers and have self-renewing capability [27,28]. Adult stem cells such as hematopoietic stem cells, bone marrow mesenchymal stem cells and neural stem cells (NSCs) are derived from specific tissues and retain the multipotent property of differentiating into major specialized cell types of the tissue [29].

The ability of stem cells offers a bright future for regenerative medicine. In 2010, the first human ESC-derived therapy product, human ESC-derived oligodendrocyte progenitor cells (OPCs) which they named as GRNOPC1, was used for

treating spinal injury [30]. Later in 2012, the first human ESC-derived terminally differentiated cells, a retinal pigment epithelium, is used to treat age-related macular degeneration and two successful results have been reported [31,32]. Nevertheless, there are still many problems in cell treatment, for instance, rejection of cells which makes it impossible to interact with the surrounding internal tissues, cells not reaching diseased site and cells not functioning as expected and possibility of harmful developments such as tumour formation from undifferentiated stem cells [33,34]. To improve the efficiency of regenerative medicine, many studies have proposed the combination of cells and materials as a solution especially cell/tissue transplantation for the damaged sites of the organ [35–38]. In addition, as mentioned in the previous sections, materials can act as stem cells regulator, dictating function of stem cells and fabricate tissue-like structure at macro and micro level [39]. Thus, we will elaborate on the roles of materials in regenerative medicine.

Bionic materials in SRM

The human body contains various tissues with different shapes and functions. Extracellular matrix (ECM) supports the survival and attachment of cells, the basic unit of an organism, in order to form functional tissues. Bionic materials are important components in tissue engineering and are used to replace damaged tissues or mimic the functions of ECM in native tissues. The materials are able to mimic natural tissues and environment partially or wholly [40]. Therefore, the research of bionics is a multidisciplinary field combining biology, chemistry, physics and materials. With hundreds of million years of evolution, organisms have the most complex structure over different ranges of scales and functions. This fact alludes to not only the importance of mimicking the biological structures, but also their functionality when designing bionic tissues or organs.

The history of bionic devices in clinical settings can be divided into three stages (Table 1). From the 1960s to 1980s, the first generation of the bionic materials was the fabrication of hard tissue-like parts such as metal bone hammer [41] and ceramic teeth [42], which are still widely used in clinical therapy to date. As synthetic technology advanced, the second generation was developed in the 1980s, which included bioceramic [43,44] and bioactive glasses [45] that could contact with physiological cues. Compared to the first generation bionic materials, there was no toxic side effect, or immune rejection response, nor disruption of the immune system, corrosion resistant and high tensile strength [46]. In the late 1990s, a new generation of biomedical materials started to be developed with biodegradable property and high specific influence on cell activities [47]. Until now, new biodegradable biomaterials are still under exploitation and they could be called as intelligent or smart materials because the materials would respond to the circumstance and regulate cell activity [48]. With these materials involved in the regenerative medicine, there is SRM development.

Natural polymeric biomaterials

Natural polymers are generally derived from native animal tissues or products, well tolerated *in vivo*, and some of them can be readily cross-linked and modified chemically. The main components of natural polymeric biomaterials are typically harvested from ECM of mammalian tissues and organs such as collagen [36,49–52], fibrin [53–55], gelatin [56–59], hyaluronic acid (HA) [60–63] and matrigel^{TM} [64–66]. Some other natural biomaterials are extracted from plant sources such as alginate [55,67–71], chitosan [72–74] and agarose [75,76]. Natural polymers, which have been used in multiple tissues and organs, have the advantages of high biocompatibility and solubility after transplantation (shown in Table 2). However, the intrinsic biological activity of natural polymeric materials may induce an immune response, transmit diseases or have uncertain efficacy [77]. Recent advances in tissue decellularization methods provide a complete ECM scaffold for detailed analysis of ECM components, organizations and biological functions [78]. The process involves dissolving and removing the cellular components of tissue by an infusion, deionized water or a mild detergent, leaving behind the

Table 1. Summary of the three generations of bionic devices.

	The first generation	The second generation	The third generation
Type	Ceramics	Bioceramics	Biomedical composite
	Metal	Bioglass	
	Alloy	Biomedical polymer material	Biomedical-derived materials
Feature	Inert material	Bioactivity or biodegradability	Bioactivity and biodegradability
Reference	[41,42]	[43–45]	[47,48]

Table 2. Summary of some properties of the natural degradable polymers discussed in this review with references on their use for tissue engineering.

Polymer	Composition	Forming methods	Application
Agarose	Consists of repeating units of alternating β-d-galactopyranosil and 3,6-anhydro-α-l-galactopyranosil groups	Thermal cross-linking	Vascular [76]
Alginate	A family of polyanionic copolymers derived from brown sea algae and comprising 1,4-linked B-D-mannuronic (M) and a-L-guluronic acid (G) residues in varying proportions	Ionic cross-linking when multivalent cations are added	Bone [71], ear [68], liver analogue [69], aortic valve [70]
Chitosan	A linear polysaccharide consisting of β-1,4 linked 2-acetamido-2-deoxy-β-d-glucopyranose units and 2-amino-2-deoxy-β-d-glucopyranose units	Easily form polyelectrolyte complexes with other polyanions	Skin [74], cartilage [182], liver analogue [55]
Collagen	Component of the ECM found in all connective tissues, which represents 30% of the total body protein in mammals	Thermal cross-linking	Bone [71], cartilage [50], ear [51], skin [36,52]
Gelatin	A mixture of peptides and proteins produced by partial hydrolysis of collagen	Thermal cross-linking, enzymatic polymerization or photo-cross-linking when treated with photosensitive groups	Bone [56], cartilage [57], liver analogue [58], aortic valve [59]
Fibrin	A natural major protein component of blood clots	Formed by the enzymatic polymerization of the protein fibrinogen in the presence of thrombin	Cartilage [50], skin [54], liver analogue [55], cardiac [53]
HA	A linear anionic polysaccharide comprising 1,3-b-Dglucuronic acid and 1,4-b-N-acetyl-D-glucosamine	Photo-cross-linking when HA treated with photosensitive groups	Skin [61], eye [63], cardiac [62]
Matrigel^{TM}	A gelatinous protein mixture derived from mouse sarcoma	Thermal cross-linking	Skin [64], aortic valve [65], vascular network [66]

tissue-specific ECM. A detailed analysis of ECM will accelerate the use of ECM scaffold in tissue engineering and regenerative medicine because the detailed information of ECM could contribute to the future high-throughput synthesis of them and would supply precise understanding of cell circumstances.

Synthetic polymeric biomaterials

Presently, wide varieties of synthetic polymers are being used in tissue engineering. Synthetic polymers are advantageous over natural materials in which they can be precisely characterized and fabricated with significant control over the physical and chemical properties [79]. Furthermore, synthetic polymers are weakly immunogenic, which is the main issue with natural materials [80]. Synthetic biomaterials have broad application in tissue engineering, whereby poly a-hydroxy acids including polyethylene glycol (PEG) [70,81–83], polylactic acid (PLA) [84,85], polyglycolide acid [86], polylactic-glycolic acid (PLGA) [64,87,88] and polycaprolactone (PCL) [89–92] are widely used. These materials share common characteristics such as high biocompatibility and degradability. However, some of them do not support cell adhesion and hence require pre-treatment with ECM or ECM-mimetic adjusting their degradation rate by modifying their structure and relative molecular weight. Nevertheless, challenges for using synthetic polymeric include the complex processing technic and lack of suitable materials with bioactivity.

Composite polymeric biomaterials

Human body comprises hard tissues, soft tissues and hard–soft tissue interfaces; therefore, materials of a single characteristic are not suitable for transplantation. On the micro level, cells are composed of well-organized polysaccharide, protein, nucleotides,

water, minerals, to name a few [93] and these cells along with ECM make up a tissue. This complexity means natural or synthetic polymer alone is not sufficient for tissue engineering. Therefore, a natural–synthetic complex has been created with the aim of improving certain properties or to create a new function with functional polymers modification or participation such as electrically conductive polymers, hydroxyapatite, metal nanoparticles and carbon nanomaterials [94]. Additionally, the biomaterials must be biocompatible and long-term stable in replacement therapy, while degradable in adjuvant therapy. In future 3D printing application, the materials must have an appropriate rheology property to promote bioprinter deposition [95]. Summarily, utilization of a combination of several materials is essential to become the pioneer of the future development.

Biomaterials for 3D bioprinting

Initially, 3D printing technology is designed for rapid prototyping using earlier materials such as metals, ceramics and thermoplastic polymers, which generally used organic solvents, high temperatures or non-biocompatible cross-linking [14]. Characterization of the physical properties (i.e. porosity, elastic modulus, degradation and water swelling) and cell-response parameters (i.e. cell viability, proliferation, differentiation and spreading) is fundamental to determine the suitability of these polymers for different tissue engineering applications [96], hence the variety of biomaterials used in tissue engineering. In this section, we will summarize the biomaterials used for solid scaffolds and hydrogels, the two main forms used for 3D printing.

Solid scaffolds are the earliest scaffolds used in tissue engineering, especially in bone substitution [4]. To date, solid scaffolds have been studied and used in regeneration of various tissues *in vitro* or *vivo*. Since the invention of stereolithography, scaffolds can be printed from inks and cured to support cell culture after printing [13]. However, the potential risks and regulatory should be fully understood before the use of solid scaffolds in clinical application. Although raw materials such as PLA, PEG and PCL have been approved by FDA, the processing unit for the industrial devices must be optimized and tested for FDA approval [97].

Hydrogels are hydrophilic polymeric network group that have been used as artificial ECM to encapsulate cells. Some biomaterials could format to hydrogels as shown in Table 2. Due to its high absorption property, hydrogels have received significant attention for cell biology and tissue engineering applications [98]. Furthermore, hydrogels possess excellent biocompatibility and possible bioactive molecules motifs encoded in their chemical structures. Hydrogels are widely used for different biomedical application, such as regeneration, drug delivery and tissue adhesives [99]. The features could allow cells to travel in the space of hydrogels and provide a room for cell growth and migration. The unobstructed diffusion of nutrients and wastes could avoid cell starvation and cell damages in the absence of vascularization [100]. However, hydrogels commonly have poor mechanical properties which make them difficult to be incorporated into devices [101]. To enhance the mechanical properties of hydrogels, nanomaterials called nanogels (monodisperse hydrogel nanoparticle), which are chemically or physically cross-linked by polymer chains to form a 3D network, are created [102]. Moreover, the concentration of encapsulated cells must be lower than the cellularity of live organs, otherwise it would limit cell proliferation and function [100]. Finally, although there are various types of printable hydrogels available, there is still no well-developed hydrogel in the market for 3D bioprinting [103].

BIOPRINTING-INVOLVED SRM

Bioprinting holds the potential to reduce the demand for donor organs as it could combine different kinds of cells and materials (either natural polymers or synthetic materials) to generate various tissues, for example heart tissue, blood vessels and cartilage, and is already in use for clinical therapy [104]. In this section, we mainly discuss the benefits of 3D bioprinting in regenerative medicine.

Bioprinting technologies

3D printing technology was first described by Charles W. Hull in 1986 [105] and has seen 30 years of development to date. It is regarded as one of 'The top ten fastest-growing production Industry in America' [106]. Presently, the use of 3D printing in various fields spans from medicine, textiles, machineries, architectural, military, jewellery to aerospace [107]. 3D bioprinting, on the other hand, is a newly emerging technology promised in the medicinal field area [108]. It is a new interdisciplinary field of regenerative medicine and tissue engineering, which combines rapid prototyping technology with biomanufacturing techniques to fabricate 3D structures, through layer-by-layer precise positioning of bioink containing biomaterials and cells. Considering the shortage of donor organs and the inevitable allograft rejection reaction, 3D bioprinting has many advantages compared to traditional tissue engineering such

Table 3. Schematics and features of selected biofabrication patterning techniques.

	Inkjet printing	Microcontact printing	Laser-assisted bioprinting
Mechanism	Non-contact, using thermal or piezo technology to prompt the liquid droplets	Contact, robotically controlled extrusion of materials	Non-contact, via pulsed laser directly onto gel with cell suspension
Print speed	1–10 000 droplets/s	10–50 μm/s	200–1600 mm/s
Resolution (μm)	>50	5–200	1–3
Cell viability	75%–90%	40%–80%	>90%
Benefits	High print speed, low cost, high resolution, wide availability	Better resolution spatial controllability and more flexibility in the material	High adaptability with materials, high cell viability, high resolution, clogging avoided, high cell concentrations
Limitations	Low droplet directionality, non-uniform droplet size, low cell concentrations, materials be liquid	Slow print speed, printer cost medium, low cell availability, low resolution	High cost, low overall flow rate
Reference	[112,183,184]	[114,185,186]	[115,120,132,187,188]

as time saving, rapid speed, high resolution, individuation and high mimicry [109,110]. The main technologies in bioprinting are inkjet [111,112], microextrusion [113,114] and laser-assisted printing [115,116]. In these systems, inks of cell suspensions are placed in a printer cartridge, where the printing patterns are controlled by a computer. However, the different features of these technologies such as surface resolution, cell viability and the biological materials used for printing can influence the quality of the products (Table 3). Each printing technology has been investigated comprehensively and has its own merits and shortcomings as illustrated in Table 3 [117–124].

Inkjet-based bioprinting is a non-contact technology. It laminates droplets of biological materials to produce 2D and 3D structures [125,126]. Currently, inkjet bioprinting mainly includes thermal inkjet and piezoelectric inkjet [127], which use thermal or piezo technology to prompt the liquid droplets, respectively. The ink in the cartridge is replaced with biological materials including cells, cell culture fluid or gel precursors. The development of inkjet printing technology is relatively of a quality and at a low cost therefore has a broad application prospects in 3D cell printing. However, the disadvantage of inkjet printing is the lack of precise control of directionality and size of droplets.

Microextrusion bioprinting is the most common 3D printing system for the printers in addition to being far more affordable. Unlike inkjet printers, which generate droplets, the microextrusion printer's materials are deposited onto a substrate. Directed by the CAD software, small beads of materials are deposited into two dimensions, where the deposited layer serves as a foundation for the subsequent layer, while the stage or microextrusion head moves along the *z*-axis, hence forming a 3D structure. The most common way to extrude biological materials for 3D bioprinting use is pneumatic [77,128,129] or mechanical (piston or screw) systems [130]. Cell-survival rate decreases with increasing extrusion pressure and nozzle gauge [131]. This observed decrease in cell viability is mainly due to the shear stresses inflicted on cells in viscous fluids. Although cell viability can be increased by using low pressures and large nozzle sizes, it also results in decrease of resolution and printing speed.

Laser-assisted bioprinting uses a laser optical tweezers effect of trace substances sink and thermal shock to deposit droplets containing cells [132]. After several decades of development, as a non-contact, sterile technology with high precision and high accuracy, the significance of laser treatment in cell therapy is recognized. According to the principles adopted by cell deposition, laser printing can be divided into two distinct technologies: laser-induced direct writing (laser-guided direct writing, LGDW) and laser-induced metastasis (Laser-induced forward transfer, LIFT). LGDW was proposed by Renn *et al.* in 1999 [133]. The laser beam may be in parallel or perpendicular orientation to generate a force in the direction so the cells would move in horizontal and vertical directions. When the force of the laser beam on the cells is greater than 10 pN, the cells may move within the range of several tens of micrometres to several millimetres, which then will be deposited on the surface of the selected object. LIFT uses laser-ablated materials as the basic of the ink. When the laser beam transferred through the transparent substrate on the interface of the thin film (transferred material) and matrix, with the interaction between

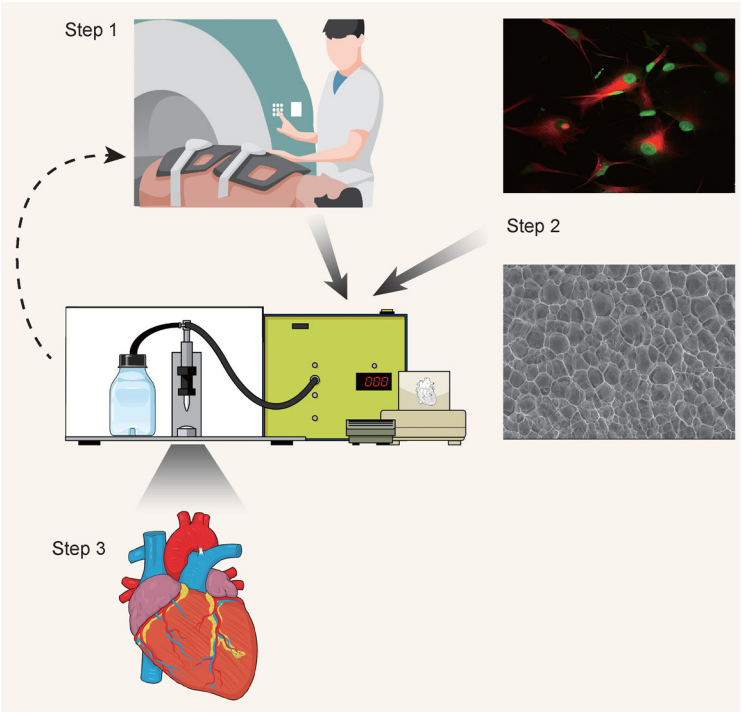

Figure 1. The process for 3D bioprinting. Step 1, imaging and model designing. Step 2, selection of cells (up) and materials (bottom). Step 3, printed 3D structure and application.

the laser and the material to be transferred (cell suspension film and material liquid), a trace amount of thin material is forced away from the base body and deposited on the bottom of the substrate-receiving layer [134].

The process of 3D bioprinting

To construct of organs using 3D bioprinting, the first step is imaging and modelling. The most common medical imaging technologies are computed tomography (CT) and magnetic resonance imaging, whereas the CAD combines the mathematical format which can assist with the modelling [14,125,135].

For the printing preparation, we have to use a bioink that is compatible with various types of cells and the matrix which can support the cell proliferation and functionalization. The material selection is based on the material's bioactivity, physical properties, biocompatibility, toxic degradation and printability. Presently, scientists can successfully extract cells from donors' bone marrow, adipose or some other tissues and then these cells could expand into sufficient numbers for the preparation of printing. Through layer-by-layer deposition from vertical to horizontal direction, the organ construction could be completed [136]. Finally, the materials should be chosen in accordance to the organ's properties.

After modelling and material selection, we need to encourage tissue fusion, remodel and accelerate tissue maturation. As organs are composed of different types of tissues, it is necessary to develop a vascular tree for the organs including capillaries and microvessels. *In vitro*, bioreactors can be used to maintain tissues and provide maturation-promoting factors. Once the organ is mature, it is ready for transplantation. The whole process of 3D bioprinting outlines in Fig. 1 [125,137].

Why does SRM need 3D bioprinting?

The goal of regenerative medicine is to develop new ways of treatment and restoration of the function for damaged and abnormal tissues. The artificial 'tissues' have primitive structures but can reach the requirements to form a 'tissue' for therapeutic use. These artificial tissues lack many typical features of biological tissues such as the complex organization of various cell types, complex ECM and microvasulature. Therefore, 3D bioprinting is needed to print tissues containing a variety of ECM, and these biological materials could be organized into uniform or non-uniform layers of complex organizational systems. In recent years, advances in 3D fabrication of biological structures include the fabrication of artificial bladders [138], skin [12], trachea [139] and heart valves [5] for clinical applications. These examples relied on thin, biodegradable scaffolds, which were filled with patients' autologous cells, hence no allograft rejections in the patients subsequently alleviating the need of organ transplantation to an extent. In the near future, 3D bioprinting will potentially play a wider role in regenerative medicine because its advantages could contribute to the thick tissue construction with imaginational structure, which has been described above.

Hard-tissue repair

Hard-tissue repair is the beginning of tissue engineering. Hard tissue is mainly composed of solids such as collagen and a substituted hydroxyapatite [140]. Therefore, acellular tissues such as bone and teeth could be repaired using biomaterials easier than the cellular tissues.

Infections, external force, abnormal bone development and other bone defects caused by diseases cause a serious impact on patient's daily life. Advance in bone tissue engineering for treating bone defects provides a new way to take advantage of the rapid prototyping technology [141,142].

Injuries occur distinctively and it requires high resolution for the interlinkage. Therefore, it is complicated to replace and repair damaged sites using traditionally fabricated scaffolds. To overcome the issue, 3D printing, one personalized technology, has started to create precise bone mould for individual patients [142]. A cancer patient, Erig Moger, whose face was mostly removed surgically, initially relied on a feeding tube to eat [143]. Later, the doctor used the CT and facial scanning technology to scan the patient's skull, followed by a construction of a normal 3D face model using 3D printing and nylon plastic. The screws for the artificial face were also created with a 3D printer. After combination with autologous bone, 3D printing could be used to treat orbital floor fractures as one cost-effective way [144]. The successful surgery of the new artificial hard tissues enhanced the quality of life of the patient greatly by enabling the patient to eat, drink and watch normally and renewed his life prospects. Similar to human bones, the structure and morphology of teeth are complex with diverse organizational structure. The dental growth and development in adapting to alveolar structure are also different; therefore, the traditional tissue engineering technology for tooth regeneration encountered several complexes [145]. At present, growth factors and autologous cells are considered to be used for hard tissue printing to enhance the tissue bioactivity [146]. There was a report showing that one thermosensitive microparticulate material combined cells to print strong constructs for bone repair [147]. These results will facilitate future cell-based hard tissue printing.

As 3D bioprinting technology is a computer-assisted modelling technology designed to meet the individual needs, the technology also has a great potential in stomatology surgery.

Soft-tissue repair

Soft tissue connects, supports or surrounds all structures or organs in the body. Traumatic injury or tumour resections often require a large amount of soft-tissue reconstruction. From the aesthetic or cosmetic point of view, soft-tissue reconstruction is also important for the patients to maintain good life quality [148]. In this section, we will review the soft tissue in skin, nervous and vascular system, which are the three main research focuses in soft-tissue regenerative medicine, where many studies have been conducted in order to reconstruct them [14].

Skin is the largest organ of the human body. Skin lost due to wounds or burns require a transplant to protect the wound, which can be difficult due to the lack of autologous or allosome skin [149]. Over the past four decades, industry and academia have invested in and designed the engineering of human skin [150–152]. Initial efforts focussed on development of a skin graft for wounds and obtained significant results. Following onto that, the research focus progressed to the development of skin model *in vitro* and the permeability of drugs and excipients [153,154]. However, the typical approach to engineering skin begins by simplifying its complexity and cannot render it to the normal layer structure. Tissue engineering has a high potential for the production of new skin. Several skin substitutes like Integra® and Matriderm® are already in use clinical settings as the supplements of autologous split-thickness skin grafts [155–157]. Nevertheless, the main question still lies in the reconstruction of subcutaneous microvascular network and sweat gland. Otherwise, 3D bioprinting could solve the problem and pattern cells as the native tissue [158]. The structure of human skin has been printed using fibroblasts and keratinocytes [159,160]; however, the development and function need future investigation [161]. Neural tissue is the main component of central nervous system and peripheral nervous system, known for their difficulty in spontaneous recovery [162]. Biological substitutes such as conductive polymers and biomateirals for the maintenance, restoration or improvement of neural tissue function have been an indispensable tool of neural regenerative medicine [163,164]. Moreover, the interaction between cells and biomaterials plays an important role in the tissue fabrication because it improves cell expansion and functionalization, which has been described in previous sections. Initially, efforts were put into the binding of the small functional molecules to scaffolds for the promotion of nerve regeneration and the results in animal models were encouraging [165]. Furthermore, cells co-cultured with biomaterials, a novel technology combination, have been used in peripheral nerve and brain injury repair [166]. Now, we have developed a novel polysaccharide-based hydrogel for NSC printing and there was functional minineural tissue construct formation [167]. To mimic the ceramal cortex structure, hand-hold printing methods were used to print primary neuronal cells and there were neuronal network observation [168].

The blood vascular system is the main transport in tissues. The main challenge in tissue engineering is the limited mass transfer [169]. Some simplex and thin tissues (i.e. skin [36], cartilage [50]) have been preliminary establishment. However, due to the size of tissue engineering, the vascular system needs to be incorporated into the tissue structure for the supply of nutrients and oxygen to cells. 3D inkjet bioprinting system was used with NIH 3T3 mouse fibroblast for tubes, which

resulted in an overhang structure with post-printing cell viability above 82% [170]. Endothelial cell derived cylindrical multicellular aggregates fabricate microvascular units. Then, a large network of blood vessels located in microvascular unit perfusion supports the self-assembly and connection to an existing network [171]. Previously, fibroblasts and umbilical vein endothelia cells combined with material scaffolds have been used for blood vessels regeneration [172]. Now, there is also a novel computer-aided algorithm and methods developed for 3D bioprinting of scaffold-free biomimetic macrovascular structures (self-supported model) [38]. To supply nutrients for the thick tissue and transport the waste, sacrificial materials such as plutonic F-127 are used in 3D printing initially, and then were removed by dissolving with fluids. The constructs retain the tube shapes for medium perfusion [173,174].

Many challenges in 3D bioprinting of tissues and organs need to be addressed. However, the preparation time for the bioink before printing is dependent on the time it takes to obtain sufficient amount of cells which is usually lengthy when using the conventional cell-culture methods, depending on the cell type [38]. In addition, materials such as synthetic or bio-based polymers are required for an extremely precise development to match with the natural tissue. The tear strength, bursting strength, mechanical and biological properties, blood compatibility and long-term stability are the primary limiting factors in creating large structures as there is no material that meets these requirements. Therefore, the field is turning its attention to combine several materials to obtain a well-rounded biomaterial for 3D printing and tissue culture or to exploring new functional materials [107].

Complicated tissues fabrication *in vitro*

Today, 3D-printed prosthetic [175], jaw bone and trachea [176,177] have been used in clinical setting with good functions. Moreover, 3D cell spheroids or hepatocytes are being used to build liver model or support artificial devices [178]. Unfortunately, there is still no solid organ that can be used for clinical transplantation, although organ models have been constructed for surgical stimulation *in vitro* with structure exactly mirroring true organs. To print organs with essential vascular network for nutrients and waste transportation, several studies have incorporated vascular tree into the printed organ and gained vascular tree constructed in the printed organ [179]. Nonetheless, there are still many challenges ahead as described above in order to generate a truly functional artificial tissue or tissue analogues.

THE IDEAL REGENERATIVE MEDICINE—SRM

As mentioned previously in the introduction, regenerative medicine holds a great promise especially with the development of prototyping technologies. However, there is still no artificial organ in the market for transplantation, mainly due to the complexity of natural organs [180]. We recommend SRM as the ideal future for regenerative medicine. Ideally, it requires a scanner to locate the injured site and a 3D bioprinter to print cells directly onto the wound to reconstruct tissues and organs *in vivo*. A scanner would first scan the patient to identify the problem, and then return with the printer heads and a mechanical arm that would perform the surgery quickly. After the operation, the machine would immediately seal the wound with a certain type of gel. This system is actually a healthcare machinery, a new technology still under development. Although this machine is still a distant future, we have already acquired its most important and basic principle which is the 3D bioprinting. Although 3D bioprinting is still in its premature stage, it has already generated several tissues at the human scale albeit with limited functions [174]. Since it is a new field in the regenerative medicine, challenges including specific techniques, vascularization, materials and cellular aspects still need to be addressed.

In 3D bioprinting technology, the compatibility with cells and physiologically relevant materials is a crucial factor in addition to high speed and resolution. The current printing materials are used either due to their compatibility with cell growth and function or their cross-linking or extrusion properties. Because of this, the range of materials for printing use are limited to collagen, HA, alginate, modified copolymers and photo-cured proteoglycans/glycoproteins (see Biomaterials for 3D bioprinting). An ideal material for bioprinting should be biocompatible, easily printed and convenient for the formation of complex 3D structures and able to maintain cellular viability and functionality, in order to provide structural and mechanical support to the overall structure. Notably, the ability to reconstruct complex 3D structures containing biologically relevant ECM proteins would be a major advance in the field.

Bioprinting also faces other challenges shared by all researchers in the fields of tissue engineering and regenerative medicine. Sources of cells for bioprinting should be readily available, easy to expand *in vitro*, non-immunogenic and biosafe. To replicate functions of the tissue or organ, it is necessary to efficiently reproduce the cell types required for specific tissues *in vitro*. Stem cells, the precursors of other

cell types, have been reported to differentiate into functional cells in the 3D microenvironment [181]. One normal tissue may contain over 1 billion cells; therefore, it is impossible to collect the large number of cells *in vitro* because of huge place, money and time required. It is also hard to their functions after printing as the cells are digested into single ones for collection and cell-cell interactions have been disrupted. If stem cells are used as the source of printing, however, they could extensively self-renew and differentiate into functional cells under defined conditions in the printed structures [167]. The oxygen, nutrient and waste transports are dependent on the cell surrounding capillary network. Therefore, vascularization is also essential for the long-term viability of any human-scale bioprinted tissue constructs.

Bioprinting tissues were studied step by step with increasing complexity, beginning with 2D tissues such as skin, to hollow tubes such as blood vessels, to hollow non-vascular tubular organs such as the bladder, and finally to solid organs such as the kidney. The increasing complexities such as cell and material requirements, tissue maturation and functionality, appropriate vascularization and innervation need to be addressed. This will be done through coordinate multidisciplinary research and the potential of 3D bioprinting can be realized, which subsequently revolutionizes the field of regenerative medicine into SRM.

ACKNOWLEDGEMENT

The authors are grateful to Siti Abdul Rahim for her grammar revision on this manuscript.

FUNDING

This work was supported by the grants from the International S&T Cooperation Programme (2013DFG30680), the National Basic Research Program of China (2011CB965000), and the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA01020101).

REFERENCES

1. Shimazono Y. The state of the international organ trade: a provisional picture based on integration of available information. *Bull World Health Organ* 2007; **85**: 955–62.
2. Mason C and Manzotti E. Regenerative medicine cell therapies: numbers of units manufactured and patients treated between 1988 and 2010. *Regen Med* 2010; **5**: 307.
3. Bello YM, Falabella AF and Eaglstein WH. Tissue-engineered skin. *Am J Clin Dermatol* 2001; **2**: 305–13.
4. Daculsi G. Biphasic calcium phosphate concept applied to artificial bone, implant coating and injectable bone substitute. *Biomaterials* 1998; **19**: 1473–8.
5. Sievers H-H. *Artificial Heart Valve*. U.S. Patent 6,991,649, Google Patents, 2006.
6. Nectow AR, Marra KG and Kaplan DL. Biomaterials for the development of peripheral nerve guidance conduits. *Tissue Eng Part B Rev* 2012; **18**: 40–50.
7. Blume SS. Histories of cochlear implantation. *Soc Sci Med* 1999; **49**: 1257–68.
8. Sifferlin A. *FDA Approves First Bionic Eye*. http://healthland.time.com/2013/02/15/fda-approves-first-bionic-eye/ (30 June 2016, date last accessed).
9. Ford E and staff. *Bionic Heart Breakthrough: Scientists Transplant Device into Sheep, Hope For Clinical Trials*. http://www.abc.net.au/news/2015-03-07/bionic-heart-breakthrough-scientists-transplant-device-in-sheep/6288126 (30 June 2016, date last accessed).
10. Jayasinghe SN. Biomaterials and bioengineering tomorrow's healthcare. *Biomatter* 2013; **3**: e24717.1–12.
11. Horst OV, Chavez MG and Jheon AH *et al*. Stem cell and biomaterials research in dental tissue engineering and regeneration. *Dent Clin N Am* 2012; **56**: 495–520.
12. Ruszczak Z. Effect of collagen matrices on dermal wound healing. *Adv Drug Deliv Rev* 2003; **55**: 1595–611.
13. Sachs E, Cima M and Williams P *et al*. Three dimensional printing: rapid tooling and prototypes directly from a CAD model. *J Manuf Sci Eng* 1992; **114**: 481–8.
14. Murphy SV and Atala A. 3D bioprinting of tissues and organs. *Nat Biotechnol* 2014; **32**: 773–85.
15. Wegst UG, Bai H and Saiz E *et al*. Bioinspired structural materials. *Nat Mater* 2015; **14**: 23–36.
16. Lytal C. *New Health Facility Aims To Translate Stem Cell Science Into Therapies*. https://news.usc.edu/62466/new-health-facility-aims-to-translate-stem-cell-science-into-treatment-therapies/ (30 June 2016, date last accessed).
17. Mason C and Dunnill P. A brief definition of regenerative medicine. *Regen Med* 2008; **3**: 1–5.
18. Atala A. Engineering organs. *Curr Opin Biotechnol* 2009; **20**: 575–92.
19. Stoick-Cooper CL, Moon RT and Weidinger G. Advances in signaling in vertebrate regeneration as a prelude to regenerative medicine. *Genes Dev* 2007; **21**: 1292–315.
20. Muneoka K, Allan CH and Yang X *et al*. Mammalian regeneration and regenerative medicine. *Birth Defects Res Part C, Embryo Today* 2008; **84**: 265–80.
21. Anonymous. Regenerative medicine glossary. *Regenerative Med* 2009; **4** (4 Suppl.): S1–88.
22. Atala A. Tissue engineering and regenerative medicine: concepts for clinical application. *Rejuvenation Res* 2004; **7**: 15–31.
23. Geiser M, Gerber P and Maye I *et al*. Retention of Teflon particles in hamster lungs: a stereological study. *J Aerosol Med* 2000; **13**: 43–55.
24. O'Neill HS, Gallagher LB and O'Sullivan J *et al*. Biomaterial-enhanced cell and drug delivery: lessons learned in the cardiac field and future perspectives. *Adv Mater* 2016, doi:10.1002/adma.201505349.

25. Olson JL, Atala A and Yoo JJ. Tissue engineering: current strategies and future directions. *Chonnam Med J* 2011; **47**: 1–13.
26. Kim BS and Mooney DJ. Development of biocompatible synthetic extracellular matrices for tissue engineering. *Trends Biotechnol* 1998; **16**: 224–30.
27. Thomson JA, Itskovitz-Eldor J and Shapiro SS *et al.* Embryonic stem cell lines derived from human blastocysts. *Science* 1998; **282**: 1145–7.
28. Takahashi K, Tanabe K and Ohnuki M *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; **131**: 861–72.
29. Kolios G and Moodley Y. Introduction to stem cells and regenerative medicine. *Respiration* 2013; **85**: 3–10.
30. Lebkowski J. GRNOPC1: the world's first embryonic stem cell-derived therapy. Interview with Jane Lebkowski. *Regenerative Med* 2011; **6** (6 Suppl.): 11–3.
31. Schwartz SD, Regillo CD and Lam BL *et al.* Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt's macular dystrophy: follow-up of two open-label phase 1/2 studies. *Lancet* 2015;**385**:509–16.
32. Song WK, Park KM and Kim HJ *et al.* Treatment of macular degeneration using embryonic stem cell-derived retinal pigment epithelium: preliminary results in Asian patients. *Stem Cell Rep* 2015; **4**: 860–72.
33. Bernstein HS and Srivastava D. Stem cell therapy for cardiac disease. *Pediatr Res* 2012; **71** (4 Pt 2): 491–9.
34. Boulis NM, Federici T and Glass JD *et al.* Translational stem cell therapy for amyotrophic lateral sclerosis. *Nat Rev Neurol* 2011; **8**: 172–6.
35. Tian H, Bharadwaj S and Liu Y *et al.* Myogenic differentiation of human bone marrow mesenchymal stem cells on a 3D nano fibrous scaffold for bladder tissue engineering. *Biomaterials* 2010; **31**: 870–7.
36. Lee V, Singh G and Trasatti JP *et al.* Design and fabrication of human skin by three-dimensional bioprinting. *Tissue Eng Part C Methods* 2014; **20**: 473–84.
37. Georgiou M, Golding JP and Loughlin AJ *et al.* Engineered neural tissue with aligned, differentiated adipose-derived stem cells promotes peripheral nerve regeneration across a critical sized defect in rat sciatic nerve. *Biomaterials* 2015; **37**: 242–51.
38. Kucukgul C, Ozler SB and Inci I *et al.* 3D bioprinting of biomimetic aortic vascular constructs with self-supporting cells. *Biotechnol Bioeng* 2015; **112**: 811–21.
39. Murphy WL, McDevitt TC and Engler AJ. Materials as stem cell regulators. *Nat Mater* 2014; **13**: 547–57.
40. Green DW. Tissue bionics: examples in biomimetic tissue engineering. *Biomed Mater* 2008; **3**: 034010.
41. Lingaraj K, Teo YH and Bergman N. The management of severe acetabular bone defects in revision hip arthroplasty using modular porous metal components. *J Bone Jt Surg Br* 2009; **91**: 1555–60.
42. Zhou R and Gao SJ. The application of post-core-metal fused ceramic crowns in esthetic restoration of the anterior teeth. *Shanghai Kou Qiang Yi Xue* 1994; **3**: 104–5.
43. Sewon LA and Keistinen AL. Review of the use of bioceramic implants in periodontal treatment. *J Ala Dent Assoc* 1990; **74**: 13–22.
44. Osborn JF and Weiss T. Hydroxylapatite ceramics–a bone-like biomaterial. Preliminary report. *Schweiz Monatsschrift fur Zahnheilkund* 1978; **88**: 1166–72.
45. Cannas M, Indemini E and Krajewski A *et al.* In vitro observations of iron-doped bioactive glasses. *Biomaterials* 1990; **11**: 281–5.
46. Hench LL. Bioactive materials: the potential for tissue regeneration. *J Biomed Mater Res* 1998; **41**: 511–8.
47. Hench LL and Polak JM. Third-generation biomedical materials. *Science* 2002; **295**: 1014–7.
48. Peppas NA and Clegg JR. The challenge to improve the response of biomaterials to the physiological environment. *Regenerative Biomater* 2016; **3**: 67–71.
49. Li N, Zhang Q and Gao S *et al.* Three-dimensional graphene foam as a biocompatible and conductive scaffold for neural stem cells. *Sci Rep* 2013; **3**: 1604.
50. Xu T, Binder KW and Albanna MZ *et al.* Hybrid printing of mechanically and biologically improved constructs for cartilage tissue engineering applications. *Biofabrication* 2013; **5**: 015001.
51. Reiffel, AJ, Kafka, C, Hernandez and KA *et al.* High-fidelity tissue engineering of patient-specific auricles for reconstruction of pediatric Microtia and other auricular deformities. *PLoS One* 2013; **8**: 8.
52. Koch L, Deiwick A and Schlie S *et al.* Skin tissue generation by laser cell printing. *Biotechnol Bioeng* 2012; **109**: 1855–63.
53. Thomson KS, Korte FS and Giachelli CM *et al.* Prevascularized microtemplated fibrin scaffolds for cardiac tissue engineering applications. *Tissue Eng Part A* 2013; **19**: 967–77.
54. Zhang Y, Cheng N and Miron R *et al.* Delivery of PDGF-B and BMP-7 by mesoporous bioglass/silk fibrin scaffolds for the repair of osteoporotic defects. *Biomaterials* 2012; **33**: 6698–708.
55. Shengjie L, Zhuo X and Xiaohong W *et al.* Direct fabrication of a hybrid cell/hydrogel construct by a double-nozzle assembling technology. *J Bioact Compat Polym* 2009; **24**: 249–65.
56. Hussain A, Takahashi K and Sonobe J *et al.* Bone regeneration of rat calvarial defect by magnesium calcium phosphate gelatin scaffolds with or without bone morphogenetic protein-2. *J Maxillofac Oral Surg* 2014; **13**: 29–35.
57. Park JH, Hong JM and Ju YM *et al.* A novel tissue-engineered trachea with a mechanical behavior similar to native trachea. *Biomaterials* 2015; **62**: 106–15.
58. Yan Y, Wang X and Pan Y *et al.* Fabrication of viable tissue-engineered constructs with 3D cell-assembly technique. *Biomaterials* 2005; **26**: 5864–71.
59. Duan B, Kapetanovic E and Hockaday LA *et al.* Three-dimensional printed trileaflet valve conduits using biological hydrogels and human valve interstitial cells. *Acta Biomater* 2014; **10**: 1836–46.
60. Burdick JA and Prestwich GD. Hyaluronic acid hydrogels for biomedical applications. *Adv Mater* 2011; **23**: H41–56.
61. Monteiro IP, Shukla A and Marques AP *et al.* Spray-assisted layer-by-layer assembly on hyaluronic acid scaffolds for skin tissue engineering. *J Biomed Mater Part Res A* 2015; **103**: 330–40.
62. Gaetani R, Feyen DA and Verhage V *et al.* Epicardial application of cardiac progenitor cells in a 3D-printed gelatin/hyaluronic acid patch preserves cardiac function after myocardial infarction. *Biomaterials* 2015; **61**: 339–48.
63. Lai JY, Cheng HY and Ma DH. Investigation of overrun-processed porous hyaluronic acid carriers in corneal endothelial tissue engineering. *PLoS One* 2015; **10**: e0136067.
64. Schumann P, Lindhorst D and von See C *et al.* Accelerating the early angiogenesis of tissue engineering constructs in vivo by the use of stem cells cultured in matrigel. *J Biomed Mater Res Part A* 2014; **102**: 1652–62.
65. Gaetani R, Doevendans PA and Metz CH *et al.* Cardiac tissue engineering using tissue printing technology and human cardiac progenitor cells. *Biomaterials* 2012; **33**: 1782–90.
66. Horvath L, Umehara Y and Jud C *et al.* Engineering an in vitro air-blood barrier by 3D bioprinting. *Sci Rep* 2015; **5**: 7974.
67. Pati F, Shim J-H and Lee J-S *et al.* 3D printing of cell-laden constructs for heterogeneous tissue regeneration. *Manuf Lett* 2013; **1**: 49–53.

68. Mannoor MS, Jiang Z and James T *et al.* 3D printed bionic ears. *Nano Lett* 2013; **13**: 2634–9.
69. Ahn SH, Lee HJ and Lee JS *et al.* A novel cell-printing method and its application to hepatogenic differentiation of human adipose stem cell-embedded mesh structures. *Sci Rep* 2015; **5**: 13427.
70. Hockaday LA, Kang KH and Colangelo NW *et al.* Rapid 3D printing of anatomically accurate and mechanically heterogeneous aortic valve hydrogel scaffolds. *Biofabrication* 2012; **4**: 035005.
71. De Coppi P, Bartsch G, Jr and Siddiqui MM *et al.* Isolation of amniotic stem cell lines with potential for therapy. *Nat Biotechnol* 2007; **25**: 100–6.
72. Walker KJ and Madihally SV. Anisotropic temperature sensitive chitosan-based injectable hydrogels mimicking cartilage matrix. *J Biomed Mater Res Part B* 2015; **103B**: 1149–60.
73. Park H, Choi B and Hu J *et al.* Injectable chitosan hyaluronic acid hydrogels for cartilage tissue engineering. *Acta Biomater* 2013; **9**: 4779–86.
74. Lin HY, Chen SH and Chang SH *et al.* Tri-layered chitosan scaffold as a potential skin substitute. *J Biomater Sci Polym Ed* 2015; **26**: 855–67.
75. Gomez-Mascaraque LG, Mendez JA and Fernandez-Gutierrez M *et al.* Oxidized dextrins as alternative crosslinking agents for polysaccharides: application to hydrogels of agarose-chitosan. *Acta Biomater* 2014; **10**: 798–811.
76. Norotte C, Marga FS and Niklason LE *et al.* Scaffold-free vascular tissue engineering using bioprinting. *Biomaterials* 2009; **30**: 5910–7.
77. Chang CC, Boland ED and Williams SK *et al.* Direct-write bioprinting three-dimensional biohybrid systems for future regenerative therapies. *J Biomed Mater Res B* 2011; **98**: 160–70.
78. Gilbert TW, Sellaro TL and Badylak SF. Decellularization of tissues and organs. *Biomaterials* 2006; **27**: 3675–83.
79. Wolf MT, Dearth CL and Sonnenberg SB *et al.* Naturally derived and synthetic scaffolds for skeletal muscle reconstruction. *Adv Drug Deliv Rev* 2015; **84**: 208–21.
80. Men Y, Gander B and Merkle HP *et al.* Induction of sustained and elevated immune responses to weakly immunogenic synthetic malarial peptides by encapsulation in biodegradable polymer microspheres. *Vaccine* 1996; **14**: 1442–50.
81. Gao G, Schilling AF and Yonezawa T *et al.* Bioactive nanoparticles stimulate bone tissue formation in bioprinted three-dimensional scaffold and human mesenchymal stem cells. *Biotechnol J* 2014; **9**: 1304–11.
82. Cui X, Breitenkamp K and Finn MG *et al.* Direct human cartilage repair using three-dimensional bioprinting technology. *Tissue Eng Part A* 2012; **18**: 1304–12.
83. Miller, JS, Stevens and KR Yang MT *et al.* Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. *Nat Mater* 2012; **11**: 7.
84. Kao CT, Lin CC and Chen YW *et al.* Poly(dopamine) coating of 3D printed poly(lactic acid) scaffolds for bone tissue engineering. *Mater Sci Eng C* 2015; **56**: 165–73.
85. Rosenzweig DH, Carelli E and Steffen T *et al.* 3D-printed ABS and PLA scaffolds for cartilage and nucleus pulposus tissue regeneration. *Int J Mol Sci* 2015; **16**: 15118–35.
86. Harenberg T, Menenakos C and Jacobi CA *et al.* Distal trachea and bronchial large lesions and suture reinforcement with Polyglicol Acid (PGA) patch. First clinical experience. *G Chir* 2010; **31**: 10–5.
87. Wei J, Han J and Zhao Y *et al.* The importance of three-dimensional scaffold structure on stemness maintenance of mouse embryonic stem cells. *Biomaterials* 2014; **35**: 7724–33.
88. Yan Q, Xiao LQ and Tan L *et al.* Controlled release of simvastatin-loaded thermo-sensitive PLGA-PEG-PLGA hydrogel for bone tissue regeneration: in vitro and in vivo characteristics. *J Biomed Mater Res Part A* 2015; **103A**: 3580–9.
89. Reichert, JC, Cipitria, A, Epari and DR *et al.* A tissue engineering solution for segmental defect regeneration in load-bearing long bones. *Sci Transl Med* 2012; **4**: 10.
90. Zopf, DA, Hollister, SJ, Nelson and ME *et al.* Bioresorbable airway splint created with a three-dimensional printer. *New Engl J Med* 2013; **368**: 2042–3.
91. Lee, CH, Rodeo, SA, Fortier and LA *et al.* Protein-releasing polymeric scaffolds induce fibrochondrocytic differentiation of endogenous cells for knee meniscus regeneration in sheep. *Sci Transl Med* 2014; **6**: 11.
92. Yeong WY, Sudarmadji N and Yu HY *et al.* Porous polycaprolactone scaffold for cardiac tissue engineering fabricated by selective laser sintering. *Acta Biomater* 2010; **6**: 2028–34.
93. Alberts B, Johnson A and Lewis J *et al.* Cell movements and the shaping of the vertebrate body. In: *Molecular Biology of The Cell*, 4th edn. New York: Garland Science, 2002.
94. Haaparanta AM, Jarvinen E and Cengiz IF *et al.* Preparation and characterization of collagen/PLA, chitosan/PLA, and collagen/chitosan/PLA hybrid scaffolds for cartilage tissue engineering. *J Mater Sci Mater Med* 2014; **25**: 1129–36.
95. Derby B. Printing and prototyping of tissues and scaffolds. *Science* 2012; **338**: 921–6.
96. Yue K, Trujillo-de Santiago G and Alvarez MM *et al.* Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels. *Biomaterials* 2015; **73**: 254–71.
97. McDermott K. Guidance document for testing biodegradable polymer implant devices (draft): division of general and restorative devices center for devices and radiological health. *U.S. Food and Drug Administration* 1996.
98. Tibbitt MW and Anseth KS. Hydrogels as extracellular matrix mimics for 3D cell culture. *Biotechnol Bioeng* 2009; **103**: 655–63.
99. Thiele J, Ma Y and Bruekers SM *et al.* 25th anniversary article: designer hydrogels for cell cultures: a materials selection guide. *Adv Mater* 2014; **26**: 125–47.
100. Guillotin B and Guillemot F. Cell patterning technologies for organotypic tissue fabrication. *Trends Biotechnol* 2011; **29**: 183–90.
101. Zhu J, Tang C and Kottke-Marchant K *et al.* Design and synthesis of biomimetic hydrogel scaffolds with controlled organization of cyclic RGD peptides. *Bioconjugate Chem* 2009; **20**: 333–9.
102. Hayashi C, Hasegawa U and Saita Y *et al.* Osteoblastic bone formation is induced by using nanogel-crosslinking hydrogel as novel scaffold for bone growth factor. *J Cell Physiol* 2009; **220**: 1–7.
103. An J, Chua CK and Yu T *et al.* Advanced nanobiomaterial strategies for the development of organized tissue engineering constructs. *Nanomedicine* 2013; **8**: 591–602.
104. Ho CMB, Ng SH and Yoon Y-J. A review on 3D printed bioimplants. *Int J Precis Eng Manuf* 2015; **16**: 1035–46.
105. Hull CW, Jr. Where we are going. *J Nurs Care* 1981; **14**: 7.
106. Setar L and MacFarland M. Top 10 fastest-growing industries. *IBIS World Special Report*. 2012; 1–4.
107. Gu Q, Hao J and Lu Y *et al.* Three-dimensional bio-printing. *Sci China Life Sci* 2015; **58**: 411–9.
108. Dieckmann C, Renner R and Milkova L *et al.* Regenerative medicine in dermatology: biomaterials, tissue engineering, stem cells, gene transfer and beyond. *Exp Dermatol* 2010; **19**: 697–706.

109. Norotte C, Marga FS and Niklason LE *et al*. Scaffold-free vascular tissue engineering using bioprinting. *Biomaterials* 2009; **30**: 5910–7.
110. Jakab K, Norotte C and Marga F *et al*. Tissue engineering by self-assembly and bio-printing of living cells. *Biofabrication* 2010: 022001 (14pp).
111. Xu T, Zhao W and Zhu JM *et al*. Complex heterogeneous tissue constructs containing multiple cell types prepared by inkjet printing technology. *Biomaterials* 2013; **34**: 130–9.
112. Xu T, Jin J and Gregory C *et al*. Inkjet printing of viable mammalian cells. *Biomaterials* 2005; **26**: 93–9.
113. Cohen DL, Malone E and Lipson H *et al*. Direct freeform fabrication of seeded hydrogels in arbitrary geometries. *Tissue Eng* 2006; **12**: 1325–35.
114. Shor L, Guceri S and Chang R *et al*. Precision extruding deposition (PED) fabrication of polycaprolactone (PCL) scaffolds for bone tissue engineering. *Biofabrication* 2009; **1**: 015003.
115. Guillemot F, Souquet A and Catros S *et al*. High-throughput laser printing of cells and biomaterials for tissue engineering. *Acta Biomater* 2010; **6**: 2494–500.
116. Guillotin B, Souquet A and Catros S *et al*. Laser assisted bioprinting of engineered tissue with high cell density and microscale organization. *Biomaterials* 2010; **31**: 7250–6.
117. Chen CS, Mrksich M and Huang S *et al*. Micropatterned surfaces for control of cell shape, position, and function. *Biotechnol Prog* 1998; **14**: 356–63.
118. Duarte Campos DF, Blaeser A and Weber M *et al*. Three-dimensional printing of stem cell-laden hydrogels submerged in a hydrophobic high-density fluid. *Biofabrication* 2013; **5**: 015003.
119. Raof NA, Schiele NR and Xie Y *et al*. The maintenance of pluripotency following laser direct-write of mouse embryonic stem cells. *Biomaterials* 2011; **32**: 1802–8.
120. Barron JA, Rosen R and Jones-Meehan J *et al*. Biological laser printing of genetically modified *Escherichia coli* for biosensor applications. *Biosens Bioelectron* 2004; **20**:246–52.
121. Gaebel R, Ma N and Liu J *et al*. Patterning human stem cells and endothelial cells with laser printing for cardiac regeneration. *Biomaterials* 2011; **32**: 9218–30.
122. Dias AD, Kingsley DM and Corr DT. Recent advances in bioprinting and applications for biosensing. *Biosensors* 2014; **4**: 111–36.
123. Gruen TA and Sarmiento A. Key references in biomaterials: bone/biomaterial interface in orthopedic joint implants. *J Biomed Mater Res* 1984; **18**: 577–99.
124. Odde, DJ, Renn and MJ. Laser-guided direct writing for applications in biotechnology. *Trends Biochem Sci* 1999; **17**: 385–9.
125. Seol YJ, Kang TY and Cho DW. Solid freeform fabrication technology applied to tissue engineering with various biomaterials. *Soft Matter* 2012; **8**: 1730–5.
126. Mironov V, Kasyanov V and Markwald RR. Organ printing: from bioprinter to organ biofabrication line. *Curr Opin Biotechnol* 2011; **22**: 667–73.
127. Boland T, Xu T and Damon B *et al*. Application of inkjet printing to tissue engineering. *Biotechnol J* 2006; **1**: 910–7.
128. Fedorovich NE, Swennen I and Girones J *et al*. Evaluation of photocrosslinked Lutrol hydrogel for tissue printing applications. *Biomacromolecules* 2009; **10**: 1689–96.
129. Chang R, Nam J and Sun W. Effects of dispensing pressure and nozzle diameter on cell survival from solid freeform fabrication-based direct cell writing. *Tissue Eng Part A* 2008; **14**: 41–8.
130. Jakab K, Damon B and Neagu A *et al*. Three-dimensional tissue constructs built by bioprinting. *Biorheology* 2006; **43**: 509–13.
131. Smith CM, Stone AL and Parkhill RL *et al*. Three-dimensional bioassembly tool for generating viable tissue-engineered constructs. *Tissue Eng* 2004; **10**: 1566–76.
132. Ovsianikov A, Gruene M and Pflaum M *et al*. Laser printing of cells into 3D scaffolds. *Biofabrication* 2010; **2**: 014104.
133. Odde DJ and Renn MJ. Laser-guided direct writing for applications in biotechnology. *Trends Biotechnol* 1999; **17**: 385–9.
134. Colina M, Duocastella M and Fernandez-Pradas JM *et al*. Laser-induced forward transfer of liquids: study of the droplet ejection process. *J Appl Phys* 2006; **99**: 084909.1–7.
135. Seol YJ, Kang HW and Lee SJ *et al*. Bioprinting technology and its applications. *Eur J Cardiothorac Surg* 2014; **46**: 342–8.
136. Blaeser A, Duarte Campos DF and Weber M *et al*. Biofabrication under fluorocarbon: a novel freeform fabrication technique to generate high aspect ratio tissue-engineered constructs. *BioRes Open Access* 2013; **2**: 374–84.
137. Lee JS, Hong JM and Jung JW *et al*. 3D printing of composite tissue with complex shape applied to ear regeneration. *Biofabrication* 2014; **6**: 024103.
138. Atala A, Bauer SB and Soker S *et al*. Tissue-engineered autologous bladders for patients needing cystoplasty. *J Urol* 2007; **177**: 66.
139. Llames S, Garcia E and Garcia V *et al*. Clinical results of an autologous engineered skin. *Cell Tissue Bank* 2006; **7**: 47–53.
140. Wahl DA and Czernuszka JT. Collagen-hydroxyapatite composites for hard tissue repair. *Eur Cells Mater* 2006; **11**: 43–56.
141. Fedorovich NE, De Wijn JR and Verbout AJ *et al*. Three-dimensional fiber deposition of cell-laden, viable, patterned constructs for bone tissue printing. *Tissue Eng Part A* 2008; **14**: 127–33.
142. Szpalski C, Wetterau M and Barr J *et al*. Bone tissue engineering: current strategies and techniques–part I: Scaffolds. *Tissue Eng B* 2012; **18**: 246–57.
143. Dailymail. *Cancer Left Eric With Half his Face Missing and Unable to Eat or Drink. Now Surgeons Have Made Him a New Face Using a 3D PRINTER.* http://www.dailymail.co.uk/health/article-2304637/Surgeon-uses-3D-printing-technology-make-cancer-patient-new-face.html#ixzz3qEaaB3yz (30 June 2016, date last accessed).
144. Vehmeijer M, van Eijnatten M and Liberton N *et al*. A novel method of orbital floor reconstruction using virtual planning, 3-dimensional printing, and autologous bone. *J Oral Maxillofac Surg* 2016, doi: 10.1016/j.joms.2016.03.044.
145. Kim K, Lee CH and Kim BK *et al*. Anatomically shaped tooth and periodontal regeneration by cell homing. *J Dent Res* 2010; **89**: 842–7.
146. Larsen M, Mishra R and Miller MJ *et al*. Bioprinting of Bone. In: Anthony Atala and James J. Yoo (ed.). *Essentials of 3D Biofabrication and Translation*. Elsevier Inc, 2015: 293–308.
147. Sawkins MJ, Mistry P and Brown BN *et al*. Cell and protein compatible 3D bioprinting of mechanically strong constructs for bone repair. *Biofabrication* 2015; **7**: 035004.
148. Hwang CM, Ay B and Kaplan DL *et al*. Assessments of injectable alginate particle-embedded fibrin hydrogels for soft tissue reconstruction. *Biomed Mater* 2013; **8**: 014105.
149. Metcalfe AD and Ferguson MW. Bioengineering skin using mechanisms of regeneration and repair. *Biomaterials* 2007; **28**: 5100–13.
150. Shevchenko RV, James SL and James SE. A review of tissue-engineered skin bioconstructs available for skin reconstruction. *J R Soc Interface* 2010; **7**: 229–58.
151. Wood FM, Kolybaba ML and Allen P. The use of cultured epithelial autograft in the treatment of major burn wounds: eleven years of clinical experience. *Burns* 2006; **32**: 538–44.

152. MacNeil S. Progress and opportunities for tissue-engineered skin. *Nature* 2007; **445**: 874–80.
153. Netzlaff F, Kaca M and Bock U *et al.* Permeability of the reconstructed human epidermis model Episkin in comparison to various human skin preparations. *Eur J Pharm Biopharm* 2007; **66**: 127–34.
154. Netzlaff F, Lehr CM and Wertz PW *et al.* The human epidermis models EpiSkin, SkinEthic and EpiDerm: an evaluation of morphology and their suitability for testing phototoxicity, irritancy, corrosivity, and substance transport. *Eur J Pharm and Biopharm* 2005; **60**: 167–78.
155. Vogt PM, Kolokythas P and Niederbichler A *et al.* Innovative wound therapy and skin substitutes for burns. *Chirurg* 2007; **78**: 335–42.
156. Supp DM and Boyce ST. Engineered skin substitutes: practices and potentials. *Clin Dermatol* 2005; **23**: 403–12.
157. Singer AJ and Clark RA. Cutaneous wound healing. *New Engl J Med* 1999; **341**: 738–46.
158. Ozbolat IT. Bioprinting scale-up tissue and organ constructs for transplantation. *Trends Biotechnol* 2015; **33**: 395–400.
159. Lee W, Debasitis JC and Lee VK *et al.* Multi-layered culture of human skin fibroblasts and keratinocytes through three-dimensional freeform fabrication. *Biomaterials* 2009; **30**: 1587–95.
160. Rimann M, Bono E and Annaheim H *et al.* Standardized 3D bioprinting of soft tissue models with human primary cells. *J Lab Autom* 2015, DOI: 10.1177/2211068214567146.
161. Vultur A, Schanstra T and Herlyn M. The promise of 3D skin and melanoma cell bioprinting. *Melanoma Res* 2016; **26**: 205–6.
162. Huebner EA and Strittmatter SM. Axon regeneration in the peripheral and central nervous systems. *Results Probl Cell Differ* 2009; **48**: 339–51.
163. Emerich DF, Orive G and Thanos C *et al.* Encapsulated cell therapy for neurodegenerative diseases: from promise to product. *Adv Drug Deliv Rev* 2014; **67–68**: 131–41.
164. Williamson A, Rivnay J and Kergoat L *et al.* Controlling epileptiform activity with organic electronic ion pumps. *Adv Mater* 2015; **27**: 3138–44.
165. Cao J, Sun C and Zhao H *et al.* The use of laminin modified linear ordered collagen scaffolds loaded with laminin-binding ciliary neurotrophic factor for sciatic nerve regeneration in rats. *Biomaterials* 2011; **32**: 3939–48.
166. Han X, Zhang W and Gu J *et al.* Accelerated postero-lateral spinal fusion by collagen scaffolds modified with engineered collagen-binding human bone morphogenetic protein-2 in rats. *PLoS One* 2014; **9**: e98480.
167. Gu Q, Tomaskovic-Crook E and Lozano R *et al.* Functional 3D neural mini-tissues from printed gel-based bioink and human neural stem cells. *Adv Healthc Mater* 2016; **5**: 1429–38.
168. Lozano R, Stevens L and Thompson BC *et al.* 3D printing of layered brain-like structures using peptide modified gellan gum substrates. *Biomaterials* 2015;**67**:264–73.
169. Lovett M, Lee K and Edwards A *et al.* Vascularization strategies for tissue engineering. *Tissue Eng B* 2009; **15**: 353–70.
170. Xu C, Chai W and Huang Y *et al.* Scaffold-free inkjet printing of three-dimensional zigzag cellular tubes. *Biotechnol Bioeng* 2012; **109**: 3152–60.
171. Jakab K, Norotte C and Marga F *et al.* Tissue engineering by self-assembly and bio-printing of living cells. *Biofabrication* 2010; **2**: 022001.
172. Kolesky DB, Truby RL and Gladman A *et al.* 3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs. *Adv Mater* 2014; **26**: 3124–30.
173. Kolesky DB, Homan KA and Skylar-Scott MA *et al.* Three-dimensional bioprinting of thick vascularized tissues. *Proc Natl Acad Sci USA* 2016; **113**: 3179–84.
174. Kang HW, Lee SJ and Ko IK *et al.* A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. *Nat Biotechnol* 2016; **34**: 312–19.
175. Alcaide-Aguirre RE, Morgenroth DC and Ferris DP. Motor control and learning with lower-limb myoelectric control in amputees. *J Rehabil Res Dev* 2013; **50**: 687–98.
176. BBC. *Transplant Jaw Made by 3D Printer*. http://www.bbc.com/news/technology-16907104 (30 June 2016, date last accessed).
177. Krassenstein B. *3D Printing Helps Create Upper Jaw Prosthetic For Cancer Patient*. http://3dprint.com/19617/osteo3d-3d-print-mouth/ (30 June 2016, date last accessed).
178. Bhatia SN, Underhill GH and Zaret KS *et al.* Cell and tissue engineering for liver disease. *Sci Transl Med* 2014; **6**: 245sr2.
179. Visconti RP, Kasyanov V and Gentile C *et al.* Towards organ printing: engineering an intra-organ branched vascular tree. *Expert Opin Biol Ther* 2010; **10**: 409–20.
180. Heidary Rouchi A and Mahdavi-Mazdeh M. Regenerative medicine in organ and tissue transplantation: shortly and practically achievable? *Int J Organ Transplant Med* 2015; **6**: 93–8.
181. Huh D, Hamilton GA and Ingber DE. From 3D cell culture to organs-on-chips. *Trends Cell Biol* 2011; **21**: 745–54.
182. Walker KJ and Madihally SV. Anisotropic temperature sensitive chitosan-based injectable hydrogels mimicking cartilage matrix. *J Biomed Mater Res B* 2015; **103**: 1149–60.
183. Xu T, Gregory CA and Molnar P *et al.* Viability and electrophysiology of neural cell structures generated by the inkjet printing method. *Biomaterials* 2006; **27**: 3580–8.
184. Xu T, Zhao W and Zhu J-M *et al.* Complex heterogeneous tissue constructs containing multiple cell types prepared by inkjet printing technology. *Biomaterials* 2013; **34**: 130–9.
185. Visser J, Peters B and Burger TJ *et al.* Biofabrication of multi-material anatomically shaped tissue constructs. *Biofabrication* 2013; **5**: 035007.
186. Jones N. Science in three dimensions: the print revolution. *Nature* 2012; **487**: 22–3.
187. Schiele NR, Corr DT and Huang Y *et al.* Laser-based direct-write techniques for cell printing. *Biofabrication* 2010; **2**: 032001.
188. Gruene M, Deiwick A and Koch L *et al.* Laser printing of stem cells for biofabrication of scaffold-free autologous grafts. *Tissue Eng Part C Methods* 2011; **17**: 79–87.