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REVIEW ARTICLE

From the printer: Potential of three-dimensional printing for orthopaedic applications

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Summary Three-dimensional (3D) printers can create complex structures based on digital models. The combination of medical diagnostic imaging with 3D printing has great potential in day-to-day clinics for patient-specific solutions and applications. In the musculoskeletal system, 3D printing is used to create custom-made implants, patient-specific instrumentation, and to regenerate tissues, in particular bone and cartilage. The major limiting factors for bioprinting include the lack of printing techniques with optimal printing resolution and materials with ideal mechanical strengths while maintaining cellular functionality. Before "tissues from the printer" can be widely applied, further research and development on improving and optimising printing techniques and biomaterials, and knowledge on the development of printed constructs into living tissues, is essential for future clinical application of this technology.

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Introduction

Over the past 30 years, there has been great advancement in medical technologies. Three-dimensional (3D) printing, a technique based on topography and photosculpture, was originally developed in 1986 by Charles W. Hull to build objects layer by layer based on digital drawings [1,2]. This technique, also known as additive manufacturing, was designed to shorten the design cycle of new products by fabricating plastic prototypes (rapid prototyping). Different kinds of materials, such as metals and ceramics, can be used for the printing of 3D objects. By using digital blueprints and image data, 3D printing has been used in various applications, such as manufacturing, the food industry, education, and art. For example, using the front and side view photographs of a person, customised prescription glasses can be 3D printed to fit personal facial features [3]. In orthodontics, x-ray images and photographs of patient's teeth can be taken or scanned using a 3D scanner. These digital images are used for treatment plans and printing orthodontics braces to align teeth [4].

The ability to use medical image data for designing a model has opened up new possibilities in the field of medicine. Three-dimensional printing can be used for patient-specific therapy, as it allows for the fabrication of custom-made implants and medical devices. In parallel, with the concept of personalised medicine, which refers to patient-specific medication based on patients' genetic profile, 3D printing can be used for personalised treatment.

In the past few years, there has been an increase in the number of publications describing the use of 3D printing techniques in patient-specific treatments. Further research in tissue engineering and regenerative medicine focus on developing specific printers and materials to create 3D constructs with living cells, growth factors, and other biomaterials using 3D printing [5]. These constructs are envisioned to replace damaged or diseased tissues and can also be used as a disease or toxicity model to study the interaction between different cell types or for drug screening. This fabrication process, also known as biofabrication, which involves the printing of living cells and biomaterials, is defined as: "the automated generation of biologically functional products with structural organisation from living cells, bioactive molecules, biomaterials, cell aggregates such as microtissues, or hybrid cell-material constructs, through bioprinting or bioassembly and subsequent tissue maturation processes" [6]. It offers the possibility to build complex tissues by the deposition of various bio-inks, such that the form and content of a construct can be tailored to the tissue to be repaired. In this article, the current techniques and recent developments of 3D printing, for orthopaedic applications in particular, is presented.

Current technologies

The general workflow of creating a 3D printed product consists of a number of subsequent steps: (1) imaging and data processing; (2) selection of printing techniques; (3) selection of materials and bioactive components; and (4) printing/bioprinting of products (Figure 1).

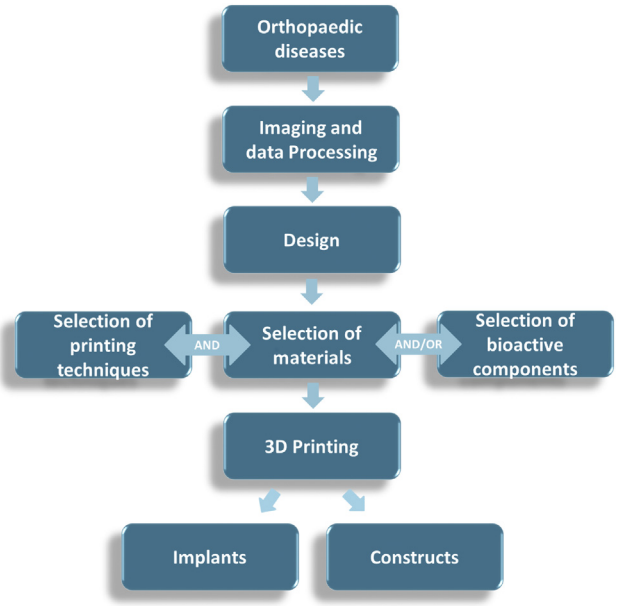

Figure 1 A schematic flow of creating three-dimensional (3D) printed products.

Imaging and data processing

Combining medical imaging and 3D printing opens up new possibilities for patient-specific therapy, as it allows for the customisation of prosthetics and implants and visualisation of complicated pathologies. The process of creating 3D models from imaging data involves image acquisition, data segmentation, and transformation into digital 3D models, followed by 3D printing and post processing [7]. The choice of imaging technique is based on the intended application and image resolution. The resolution of the resulting image is important, as images with poor resolution will result in an inaccurate and unfit model. In orthopaedics, imaging techniques such as x-ray imaging, computed tomography (CT), and magnetic resonance imaging (MRI) are commonly used. X-ray imaging and CT are often employed to diagnose bone fractures or muscle disorders, whereas MRI is used to detect soft tissue damage. Once the initial imaging data has been acquired, further processing, which includes selecting and isolating the region of interest using open or proprietary software, and transforming segmented data into volumetric data, will be carried out prior to printing.

Printing techniques

In order to meet the intended applications of a scaffold or implant, the architectural design of a construct at various levels, macro- (overall shape), micro- (tissue architecture) and nano-scales (surface modification) is important [8]. The selection of a 3D printer depends highly on the materials of interest and resolution of the products. Common printing techniques include fused deposition modelling (FDM), selective laser sintering (SLS), and inkjet printing. FDM printers generally extrude materials that are heated at the

nozzle and harden gradually after extrusion. As the print head moves, it builds objects in thin layers. This cycle of printing repeats until a solid 3D object forms. SLS uses a laser as an energy source and draws the shape of an object to sinter powdered material. A new layer of material applies on top, and the process repeats until the part is completed. Inkjet printing uses thermal, air pressure, electromagnetic, or piezoelectric technology to dispense droplets of ink onto a substrate. By changing the applied temperature gradient, pressure, pulse frequency, and ink viscosity, the droplet size can be modified for different applications. Based on the versatility, precision and speed of the printers, and the availability of materials, these printing techniques have been used to print objects for different applications.

Materials

Each material for 3D printing has its specific mechanical properties, processing methods, chemical properties, and cell-material interactions. Some of the commonly used materials include metals, bioceramics, synthetic, and natural polymers (Figure 2). Metals and bioceramics are mainly employed to create implants and for bone restoration. For implantation, titanium (Ti) and its alloys have been demonstrated to be biocompatible with good mechanical properties [9]. Bioceramics, such as hydroxyapatite (HA), calcium phosphate, and bioglass, have been used for bone regeneration, as they are osteogenic, porous, maintain their shape, and promote cell proliferation on their surfaces. However, these materials lack appropriate mechanical strength for implantation in load-bearing sites [10,11]. Recently, scaffolds based on composite materials, such as HA and tricalcium phosphate (TCP) [12—14], polycaprolactone (PCL)-HA with carbon nanotubes [15], PCL-poly lactic-co-glycolic acid (PLGA) [16—19], and PLGA-TCP [20—22] have been investigated as scaffold materials in order to optimize the architecture, biocompatibility, and sintering conditions (particle size and sintering temperature) to improve the porosity, mechanical strengths, and biocompatibility of these constructs. Hydrogels, another important class of biomaterials, are commonly used as cell carriers in tissue engineering. Hydrogels are designed to act as an artificial extracellular matrix and provide living cells a 3D environment to grow. The combination of hydrogel with cells and/or growth factors functions as a bio-ink. The type of polymer, chemical composition, molecular weight, and concentration of hydrogel directly determines the viscosity, speed of gelation, and mechanical strength of the scaffold.

The optimal printing fidelity (shape, complexity, and resolution of the construct) will be determined by the processing parameters, including the fabrication time and nozzle gauge, which in turn, will affect the cell viability and function [5]. Therefore, materials should be carefully chosen based on the intended application of the construct. Aspects, such as the mechanical strength of the materials and structural requirements of the constructs are essential, as these vary among the diverse target tissues. The ultimate goal is to mimic the structure and mechanical properties of the native/target tissue and develop the printed constructs into a functional tissue. However, the lack of printable and regulatory bodies approved materials, suitable bio-inks, and lengthy production time limits the development of bioprinting for clinical use. Therefore, the current development of materials for bioprinting aims to solve these problems.

Current applications in orthopaedics

Three-dimensional printing offers a range of possibilities for patient-specific therapy. In orthopaedics, 3D printing has been applied in various aspects including designing and printing customised equipment for surgery, printing implants, and prostheses for support, and regenerating musculoskeletal tissues including bone, cartilage, and soft tissues such as tendon, ligament, and muscles.

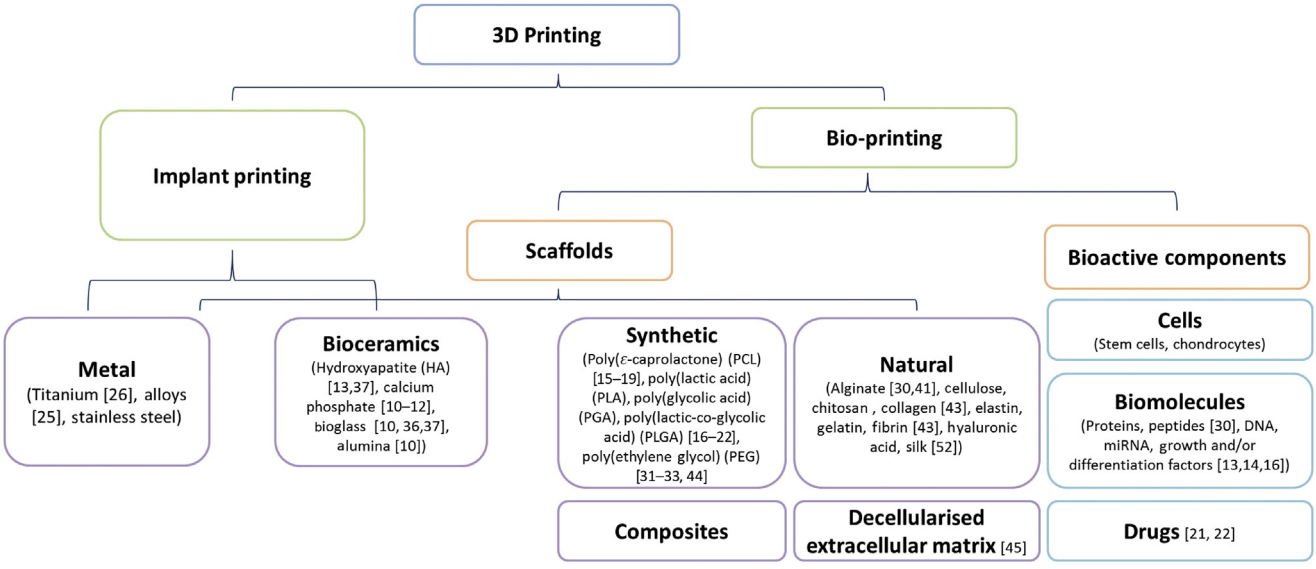

Figure 2 Materials commonly used in three-dimensional (3D) printing and bioprinting.

Customised surgical assistive tools and implants

Three-dimensional printing is a suitable technique to create patient-specific anatomical models, customised moulds, and surgical guides, as well as permanent implants. Some of the many benefits of employing 3D printing are that it allows better surgical planning, creates customised patient-specific implants, shortens surgical time, and hospital stay. It also reduces morbidity, yields better fitting of prosthesis, as well as better educates and enhances patients' understanding of surgical procedures. Many of the patient-specific models, guides, and templates are routinely used (Figure 3). For example, patient-specific 3D printed screw guide template systems can benefit intraoperative pedicle screw fixation, a standard procedure of spinal instrumentation, in the thoracic spine by improving the accuracy of the surgical procedure, reducing the operating time and radiation exposure during the surgery [23]. Furthermore, patient-specific, disposable surgical saw guides/cutting-blocks can be printed for the use in total knee arthroplasty. The use of imaging and planning software, the different cuts of the bone resections, and the size and position of the knee implant can be planned prior to surgery. Each patient-specific saw guide/cutting-block provides guidance to surgeons during surgery, and reduces the number of decisions he/she has to make during surgery. This can minimize tissue loss and optimise the positioning of implants, and hence, lengthen the lifetime of the prosthetics [24]. With the use of patient-specific surgical assistive tools, the risk of surgical errors and outliers can be reduced, and operations will be less dependent on the experience of the physician.

In addition to surgical assistive tools, implants have also been printed for treating orthopaedic diseases. For example, a 3D printed customised axial vertebral body has recently been implanted in the upper cervical spine of a patient [25]. One case study has also employed 3D printing to create a titanium calcaneal prosthesis for a patient who has chondrosarcoma in the heel [26]. These cases demonstrate how 3D printed, patient-specific implants may bring individualised solutions to rare and complicated problems, where restoration of the specific anatomy of each patient remains challenging.

Musculoskeletal tissues

Three-dimensional printing approaches that aim to solve various musculoskeletal tissue diseases are currently under development and are most frequently studied *in vitro* for bone and cartilage regeneration, and fewer for meniscus, tendon, ligament, and muscle regeneration. As 3D bioprinting has not reached the clinic yet, the following sections will describe and review the current trends in the application of 3D printing for musculoskeletal tissue regeneration.

Bone regeneration

Bone is a dynamic tissue, with the ability to self-regenerate and self-repair. However, cancer, trauma, infection, and congenital deformity can lead to massive bone defects or

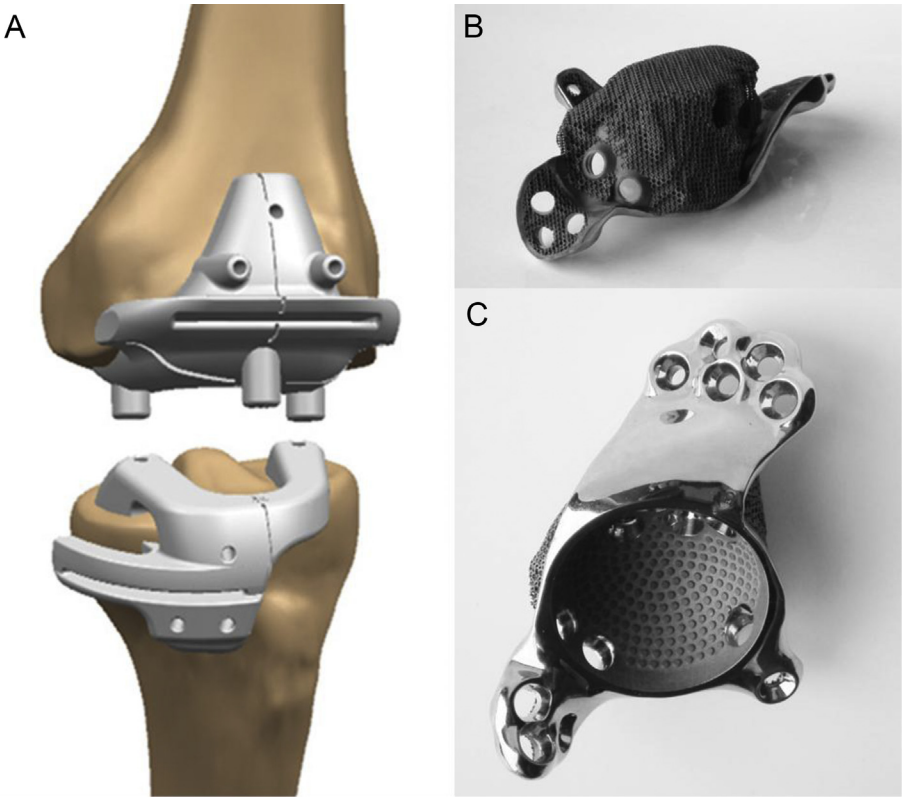

Figure 3 (A) Patient-specific sawguides for total knee arthroplasty (Smith & Nephew, USA) and (B, C) custom-made titanium acetabulum implant with screw planning (Materialise, Belgium).

loss that fails to heal. Transplantation of vascularised autologous bone grafts (autografts, allografts, and artificial bone substitutes), which promotes bone healing through osteogenesis, osteoinduction, and osteoconduction, have been used for this purpose [27]. However, some of the major limitations of these grafts include donor site co-morbidity, rejection, poor graft incorporation, and transmission of disease [28]. Therefore, tissue engineering, which uses scaffolds and combinations of cells, materials, and/or biologics to improve or replace biological tissues, is an attractive strategy for bone regeneration. Some of the essential elements of an effective bone scaffold include the use of biocompatible and bioactive materials, and a porous 3D matrix that allows cell attachment, delivery of nutrients to cells and cell migration, and the ingrowth of blood vessels. In addition, the scaffold must possess a suitable mechanical stability, flexibility and allow the transfer and diffusion of growth and differentiation factors [29]. For cell viability and tissue maturation within a gel, covalent immobilisation of extracellular matrix (ECM) molecules or adhesive peptides can be used. For example, the presence of a peptide such as arginine—glycine—aspartic acid significantly increases the amount of bone formation in an alginate scaffold. In addition, the delivery of multiple growth factors has also been demonstrated to improve bone tissue formation in alginate gels [30]. In addition, polyethlyene glycol (PEG) hydrogels have been used extensively as mechanically strong, cytocompatible matrices, which can maintain cell viability and promote ECM production [31—33]. Because 3D printing allows a precise control of the overall geometry and the internal porous structure of a scaffold, much of the current research on bone regenerative medicine utilises this versatile technique. One recent publication studied the biocompatibility of 3D printed calcined bone—biphasic ceramic composite/PVA gel both *in vitro* and *in vivo*. The scaffold showed good mechanical properties, and the rabbit's bone marrow stromal cells grew, proliferated, and differentiated on the surface of the scaffold after adherence. *In vivo* experiments also demonstrated that the bone scaffolds showed high biocompatibility [34]. One study introduced osteoinductive compounds, silica (SiO_2), and zinc oxide (ZnO), to β-TCP, a known osteoconductive material, and were 3D printed to investigate their osteoinduction potential *in vivo*. The results of this study showed that the combination of SiO_2 and ZnO dopants in TCP maybe a viable alternative to introduce osteoinductive properties to calcium phosphates (CaPs) [35]. Other novel approaches in bioprinting combined human mesenchymal stem cells (hMSCs) and poly(ethylene) glycol dimethacrylate (PEGDMA) with bioactive glass and/or HA to form the homogeneous bone constructs in a layer-by-layer approach. Significantly higher total collagen production and alkaline phosphatase (ALP) activity in hMSCs were observed within the printed scaffold. This higher collagen production was also observed in previous studies, and the presence of HA increased the ALP activity in hMSCs and promoted osteogenesis [36,37]. Future research directions include exploring and expanding the current group of biomaterials for bone regeneration, as well as studying the stability of the individually designed blocks, potentially with incorporated prevascular networks for larger bone replacement without an external matrix for support.

Cartilage regeneration

Cartilage degeneration due to age or injuries is one of the most common musculoskeletal problems. Articular cartilage, which lines all the articular joints in the body, provides a smooth, lubricated surface and mechanical support of joint movements [38]. The articular cartilage, a hyaline cartilage, has a unique composition and is comprised of an extracellular matrix composed of water, collagen, proteoglycans, noncollagenous proteins and glycoproteins, and highly specialized chondrocytes which contribute to four zones of cartilage, the superficial, middle, deep, and calcified zone [38]. The articular cartilage is avascular, lacks innervation and a lymphatic system, and is subjected to a harsh biochemical environment in the intra-articular space. With the absence of blood flow, the articular cartilage has a limited capacity for intrinsic healing and repair. Very often, untreated cartilage injuries will progress to arthritis of the joint, which currently has no cure. Bioprinting can create constructs, which mimics the architecture of tissues to be repaired, and provides a potential treatment modality for cartilage repair [39]. Besides, the size, depth, and strength of the construct can be monitored closely. Before the implantation of the tissue into the body, the bioprinted construct needs to grow in a controlled environment (bioreactor) into a functional tissue. The maturation will then take place *in vivo* and the construct/tissue can integrate into the host body/tissue. Another possibility would be to integrate the functions of the tissue directly into the printed construct. For example, one study has investigated the fabrication of cell-laden, heterogeneous hydrogel constructs using 3D printing for the potential use as osteochondral grafts. Both osteogenic progenitors and chondrocytes were encapsulated in different parts of the construct, and the study demonstrated that the anticipated tissue type were formed [40]. A similar study attempted to build osteochondral constructs using two different materials, PCL and alginate, and encapsulate with osteoblasts and chondrocytes. This study was able to create a dual cell-laden scaffold, with enhanced mechanical properties while maintaining the anatomical position and viability of cells [41].

The current development of bioprinting of cartilage tissue constructs includes improving the mechanical strength of hydrogels by coupling with synthetic polymers, creating zone specific cartilage constructs and creating osteochondral plugs that include both the cartilage and bone compartments [42]. For example, PCL fibres and chondrocytes suspended in a fibrin—collagen hydrogel have been printed to create a cartilage construct. The study demonstrated that the constructs formed cartilage-like tissues both *in vitro* and *in vivo*, and with enhanced mechanical properties [43]. To create zone specific cartilage, multilayered constructs composed of various combinations of polymers, including PEG, hyaluronic acid, chondroitin sulphate, and metalloprotease sensitive peptides were used to encapsulate a single lineage of mesenchymal stem cells. The study was able to create a single construct, which comprised of all three zones of articular cartilage from one single stem cell lineage [44].

One of the obstacles of using the current biomaterials for cartilage repair is that there is limited cell-material interactions and often forms inferior tissues [45]. Besides,

the available synthetic or natural biomaterials are unable to mimic the complexity of natural extracellular matrix and its intrinsic functions. Recently, decellularised ECM (dECM) has been printed to provide microenvironments that induce the growth of cartilage tissue. This study demonstrated that dECM provides crucial signals for cells engraftment, survival and its long-term function [45]. Therefore, in order to create constructs for optimal cartilage regeneration, the mechanical strength, cell survival, and functionality are equally important.

Meniscus regeneration

Meniscus in the knee is a fibrocartilaginous tissue, which acts as a shock absorber to protect the articular cartilage during knee movements and stabilize the knee. Similar to articular cartilage, the meniscus has a heterogeneous composition of connective tissue cells including the fibroblast-like cells, fibrochondrocytes and chondrocyte-like cells, and components such as collagen type I, II, and glycosaminoglycans [46]. The meniscus also has minimal blood flow; the central region is avascular and therefore, fails to heal. The current surgical treatments for meniscal injuries include total and partial meniscectomy, meniscal repair, and meniscal transplantation. Meniscus allografts have been demonstrated to provide short-term benefits in young patients, however, the durability and ability to reduce the risk of progression of osteoarthritis of these allograft is still unknown.

Tissue-engineering approaches have been investigated for meniscus regeneration. Previous work on meniscus regeneration aimed to mimic the structure, mechanical properties, and improve the integration of meniscal scaffolds using various combinations of biomaterials and cells. For example, scaffolds, made of polyurethane, showed optimal mechanical properties with interconnective macroporosity to facilitate cell ingrowth and differentiation [47]. One study utilized a 3D printing technique, projection stereolithography, to simulate the structural architecture of meniscus, and the scaffold was seeded with human cells from the meniscus. This study demonstrated that cells were able to grow with an organised cellular alignment and promote meniscus-like tissue formation [48]. In addition, one recent study was able to mimic the zone-specific matrix phenotypes of meniscus in 3D printed scaffolds incorporated with spatiotemporally delivered human connective tissue growth factor and transforming growth factor−β3. These implants were placed in sheep, and the regenerated meniscus demonstrated the ability to restore the mechanical integrity of the meniscus [49].

Tendon, ligament, and muscle regeneration

In addition to bone, cartilage, and meniscus, the musculoskeletal system is also made up of muscles and connective tissues including tendon and ligament, which are structurally optimised to generate and transfer force, and facilitate movements. Similar to bone and cartilage, tissue engineering using 3D printing techniques have been employed to mimic and create functional muscles, tendons, and ligaments. Large skeletal muscle tissue defects can be due to trauma, tumours, and congenital conditions. The current treatment options are limited by the availability of host tissues, as well as donor site morbidity. In one study, human skeletal muscle cells were seeded onto a scaffold, which was created by electrospinning. The results showed that the orientation of the nanofibres can significantly affect the induction of muscle cell alignment and myotube formation, and that the aligned composite nanofibre is promising for future functional muscle tissue implantation [50]. Taking this one step further, researchers have also begun to investigate fabricating tissues as a functional, composite unit, for example a muscle-tendon unit and ligament-bone unit. One study has specifically attempted to create a region-specific scaffold, using two synthetic polymeric materials and two cell-laden hydrogel based bioinks, to mimic the mechanical and biological properties of muscles and tendons [51]. In addition, an *in vivo* study employed 3D printing to create a ligament-bone composite scaffold with an aim to aid ligament reconstruction surgeries. The ceramic bone scaffold was created using 3D printed resin moulds, and the ligament scaffold was created by weaving degummed silk fibres. The study demonstrated that there was a significant difference in mechanical strengths, new bone formation in the bone scaffolds, as well as a gradual structural transition between the scaffolds and host bones. This signified that the ligament-bone composite scaffolds was able to facilitate the regeneration of tissues at the ligament−bone interface [52].

Challenges and future directions in bioprinting

With the ability to further mimic the cellular and extracellular structure and components of a tissues and organs, 3D printing possesses significant potential in regenerative medicine. However, there are challenges and limitations in every step of creating a biological construct, from printing techniques to materials and cell sources. Although significant steps have been taken, further optimisation of bioinks, fabrication time, and biological performance would be necessary in order to bring 3D bioprinting to the clinic. There is currently a limited range of biomaterials available for bioprinting, as most publications are using very similar materials [5]. Therefore, there is a need to investigate and develop a diverse selection of materials that are biocompatible, mechanically supportive and can maintain cell viability and functions for 3D (bio)printing. In addition, similar to any organ or tissue transplantation, there is always a chance of rejection of bioprinted constructs by the host immune system. Autologous and allogenic (stem) cells and induced pluripotent stem cells are alternative cell sources; however, research on their safety will need to be further verified. Furthermore, the maturation of cells, vasculature and innervation are common challenges for the bioprinting of larger tissue constructs. Vascularisation is essential for the long-term viability of cells. A recent review has suggested using a bioreactor for vascularisation, as it can maintain the viability of a tissue construct while allowing further processing. For example, using bioreactor processing in combination with factors that promote angiogenesis and innervation can maintain cell viability [53].

Three-dimensional printed constructs, such as other medical devices, are subjected to regulatory approval prior to commercialisation. Currently, 3D printed devices are subjected to the same regulations listed in Section 510(k) of

the Food, Drug, and Cosmetic Act from the United States (US) Food and Drug Administration (FDA). However, devices created by 3D printing may require different or additional testing, as they go through different manufacturing techniques and consist of various parts such as materials, drugs, and cells, compared with traditional techniques. Most printed implants that have been applied in patients were either used for improving surgical precision, for example, a 3D printed titanium bone tether plate has been granted FDA clearance to preserve bone anatomy [54], or were granted emergency use such as 3D printed splints implanted in babies with severe tracheobronchomalacia [55]. The FDA is working towards issuing guidance on 3D printing, and a public workshop on additive manufacturing of medical devices was held in October 2014 to discuss medical considerations of 3D printing [56].

Although 3D printed metal implants, (cell-free) biocompatible plastic materials and or constructs as carriers and custom-made devices are already available in clinical settings, bioprinting is still in its infancy and far from clinical applicability. The organised printing of cells and biomaterials will, in the short term, be primarily used as a test model in research settings. The challenges and limitations mentioned are still restricting the clinical implantation of living printed constructs. To obtain a functional 3D printed tissue, the organisation of the printed construct, the environment for the optimal tissue maturation, and the printing techniques should be further optimised. After overcoming the technical limitations and proving clinical effectiveness, there will be more applications of "tissues from the printer".

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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