REVIEW ARTICLE

The trend towards *in vivo* bioprinting

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Abstract: Bioprinting is one of several newly emerged tissue engineering strategies that hold great promise in alleviating of organ shortage crisis. To date, a range of living biological constructs have already been fabricated *in vitro* using this technology. However, an *in vitro* approach may have several intrinsic limitations regarding its clinical applicability in some cases. A possible solution is *in vivo* bioprinting, in which the *de novo* tissues/organs are to be directly fabricated and positioned at the damaged site in the living body. This strategy would be particularly effective in the treatment of tissues/organs that can be safely arrested and immobilized during bioprinting. Proof-of-concept studies on *in vivo* bioprinting have been reported recently, on the basis of which this paper reviews the current state-of-the-art bioprinting technologies with a particular focus on their advantages and challenges for the *in vivo* application.

Keywords: bioprinting, *in vivo*, tissue engineering, regenerative medicine

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1. Introduction

It is almost certain that the traditional organ transplantation approach can never meet the mounting global demands for tissue/organ replacement. One of the most promising solutions to the organ shortage crisis is tissue engineering (TE). Bioprinting, as one of several newly emerging TE strategies, is an attractive additive manufacturing-based biotechnology which uses "bioinks" such as living cells, tissue spheroids, and bio-hydrogels to spatially construct 3D functional structures without pre-fabricated scaffolds. The ultimate goal of bioprinting is to *de novo* synthesize tissues/organs that are suitable for complete restoration of degenerated, damaged or lost ones.

Like most newly emerging biotechnologies, bioprinting was first introduced as an *in vitro* technique in the laboratory[1]. To date, laser-based, inkjet-based and nozzle-based bioprinting systems are under parallel development, the majority of which are for *in vitro* strategies, in which tissue/organ constructs are prefabricated and cultured *in vitro* prior to implantation[2–5]. However, *in vitro* bioprinting may have several intrinsic limitations regarding its clinical applicability. Firstly, even after setting aside several ethical issues which may limit the application of *in vitro* tissue/organ culture techniques, there is still a long way before such technology becomes sophisticated and efficient enough to synthesize functional tissue/organ replacements outside the body. Secondly, bioprinted tissue/organ substitutes normally have weak initial mechanical strengths due to the fluid-rich nature of the biomaterials used, hence handling and fixation of such fragile living constructs to equally sensitive neighboring native tissues, along with strict sterile requirements on the entire routine (from *in vitro* fabrication,

culture, and transportation to *in vivo* implantation), can lead to low replication rates and low error tolerance rates, which may make *in vitro* bioprinting-based therapeutic strategies less attractive to surgeons. Thirdly, it is possible that the shape/morphology of the prefabricated construct may not match the actual size of the defect, which results in not only prolonged surgical time due to further construct-trimming or gap-filling, but also a compromised mechanical anchoring of the construct to the native tissues. In addition, the surgical implantation procedure can be destructive to the prefabricated tissue/organ substitutes, i.e., the physical impact of the fixation process when pressing or suturing the *in vitro* printed construct to the peripheral native tissues may disrupt both the micro- and macro-architectures of the substitute, resulting in noticeable impairment to the structural integrity, cell viability, and initial bridging of the implant to its surroundings.

A possible solution to the aforementioned *in vitro* issues is to directly fabricate and position tissue/organ substitutes at the defective site in the living body, which is the so-called "*in vivo*" bioprinting, a term occasionally interchangeable with "*in situ*" bioprinting. In a typical *in vivo* bioprinting scenario following the real-time surgical schemes, robotic arms with bioprinting units enter the body through minimally invasive route and automatically reconstruct new tissues/organs with hierarchical and physiological equivalence to the originals under the control of surgeons. In this way, the human body functions as the effective "*in vivo* bioreactor" to facilitate maturation of the printed constructs in a real biological environment right from the fabrication stage. *In vivo* bioprinting would be particularly effective in the treatment of tissues/organs that can be safely arrested and immobilized during bioprinting, e.g., the musculoskeletal system. Success in such a fascinating technology will completely revolutionize surgical practice in the future. Rather than being a publicity stunt, *in vivo* bioprinting is now a promising work in progress. To push this inspiring but tough work forward, it is logical to start with superficial tissues/organs which can be accessed easily. In this review, we will give an overview of the state-of-the-art *in vivo* bioprinting researches carried out worldwide, and advances in biofabrication and biomaterial technologies with a particular focus on the potential for the eventual realization of *in vivo* bioprinting in the future.

2. Current Status of *in vivo* Bioprinting

As the largest and most easily accessible organ in the body, the skin has logically become the ideal place to start with for the development of *in situ* bioprinting. Binder *et al*. demonstrated their *in situ* inkjet-based 3D skin printer with which they directly repaired the skin defects on rats[6, 7]. The results indicated that multiple skin cells could be directly delivered onto a wound with an acceptable cell survival rate through the *in situ* printing procedure. The wounded skin also exhibited faster recovery after being printed with skin cells. Sofokleous *et al*. brought up the concept of using a portable handheld device to deliver drug, construct artificial skin, and apply wound dressing patch directly onto the damaged skin[8]. This portable electro-hydrodynamic device demonstrated not only a co-axial multi-needle design for integral fiber construction with multiple diameters, but is also a good example of bringing bioprinting techniques out of the laboratory and towards practical applications, i.e., from *in vitro* to *in vivo*.

Another branch of pilot studies deal with *in vivo* repair of osteochondral tissues, i.e., the bone and articular cartilage. Keriquel *et al*. applied high-throughput biological laser bioprinting technologies to repair mouse calvaria defects of critical size (3 mm in diameter, 600 μm in depth) through deposition of nano-hydroxyapatite (n-HA) *in vivo*[9] as shown in Figure 1. Preliminary results from this study, such as no detectable deleterious effects of infrared laser light on brain tissues; satisfying morphological, chemical and biological properties of the *in vivo* printed construct; and recovery achieved in most cases (29 out of 30 cases, indicated the feasibility of *in vivo* bioprinting for repairing superficial osseous defects. Cohen *et al*. demonstrated the *in situ* repair of both chondral and osteochondral defects on *ex vivo* excised bovine femoral condyles using a built-in-house bioprinter (Figure 2)[10]. The demineralized bone matrices and alginate hydrogels were sequentially printed onto the osteochondral defect, to rebuild bone and cartilage respectively. A meaningful attempt in this pilot study was the establishment of a novel geometric feedback system, through which the *in situ* bioprinting system boasted a precision of mean surface errors of less than 0.1 mm.

Moreira Teixeira *et al*. proposed an arthroscopy-compatible extrusion-based approach to repair small articular cartilage (AC) defects, by filling the AC

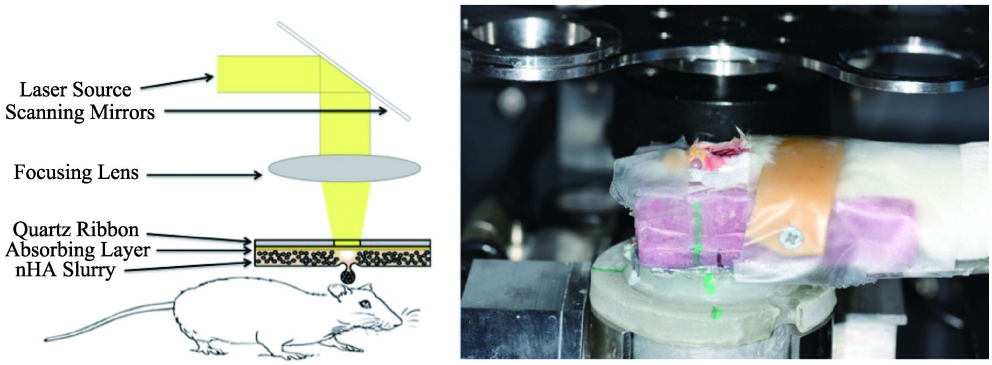

Figure 1. A laser induced forward transfer (LIFT)-based bioprinting system that Keriquel *et al*.[9] developed for *in vivo* repair of mouse calvaria defects. (The images[9] are reused with permission from © IOP Publishing. All rights reserved.)

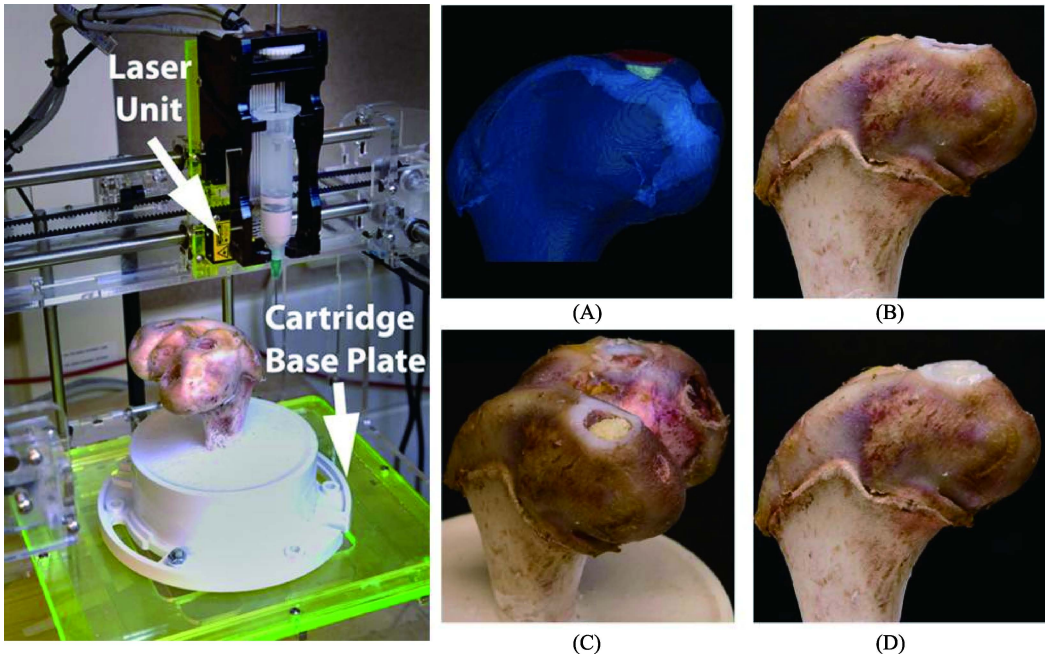

Figure 2. The robocasting nozzle-based bioprinting system developed on the basis of a Fab@Home open-source, open-architecture AM system. The laser highlighted in the left image was used for measuring the geometric fidelity of printed constructs. Images of resultant print geometry: (A) CT image of the bovine femur with an osteochondral defect (cartilage defect highlighted in red, bone defect in yellow); (B) Photo of the femur with an osteochondral defect; (C) Bone portion has been printed; (D) Alginate hydrogel has been printed on the bone portion as a cartilage cap. (Image[10] courtesy of Daniel Cohen, Cornell University and reproduced from with permission from © IOP Publishing. All rights reserved.)

defects with a cell-free injectable hydrogel comprising dextran-tyramine conjugates (Dex-TA)[11]. A handheld device named "BioPen", has recently been developed in the laboratories of the University of Wollongong for *in vivo* repair of osteochondral defects. By combination of nozzle-based material delivery system and a low-powered ultraviolet light curing system, this technique allows operators to control the deposition of stem cell-loaded materials to create customized implants as they work[12]. *In vivo* reconstruction of biological tissue has also been achieved in corneal regenerative medicine using an *in situ* formable hydrogel[13]. It is understandably arguable whether some of the aforementioned *in situ* tissue repair techniques based on handheld syringes can be properly categorized as bioprinting since the "precise 3D printing" feature is diluted. However, advances in these endoscopy-compatible techniques for *in situ* tissue organ repair will undoubtedly be part of the foundations of *in vivo* bioprinting.

To date there is still no printed tissue that can satisfactorily mimic full hierarchical structures and func-

tions of natural tissues such as sweat glands in the skin. This reflects the fact that quite a few challenges facing current bioprinting techniques remain unsolved. For *in vivo* bioprinting, even more challenges arise when adapting knowledge gained through *in vitro* systems and the repair of superficial tissues/organs to the *in vivo* repair of internal organs. Firstly, the size of the printing units must be minimized to comply with the increasingly-popular minimally invasive surgical techniques. This can be extremely challenging for some biofabrication techniques whose setups require large amounts of external instruments. Secondly, the demands of *in vivo* environments of internal tissues/ organs are complex and may not be conducive to formation of existing biomaterial constructs due to high moisture, high oxygen level, and dramatic differences in mechanical and chemical properties of adjacent native tissues, which together pose new challenges to materials science. In addition to satisfying strict requirements on biocompatibility and biodegradability for biomaterials, materials which are suitable for *in vivo* bioprinting should have a rapid polymerization and crosslinking rate (ideally a few seconds to minutes), and a capability for polymerization and crosslinking under a moist, oxygen-rich condition. Last but not least, transferring bioprinting from laboratory to operating room requires the synchronous advancement of surgical technologies, particularly robot-assisted operation systems which allow precise micro-manipulation inside the body, to provide reliable control over *in vivo* biofabrication.

The development of *in vivo* bioprinting therefore involves cell biology, materials science, biofabrication, and surgical technologies. In brief, a deep understanding of cell behaviors forms the foundation for guiding *in vivo* cell proliferation and functional extracellular matrix (ECM) formation. Development of sophisticated biomaterials, which support cells with physiochemically and biologically satisfying environments while facilitating fast bioprinting, will play pivotal roles. Advanced biofabrication technologies provide effective tools to realize the controllable construction of new tissues/organs at the desired site. Novel surgical technologies, which allow precise control of bioprinting devices in the operating room, are final steps towards clinical application. In the next few sections, we will review the state-of-the-art and technical trends in biofabrication, biomaterials, surgical technologies and their future applications to *in vivo* bioprinting.

3. Challenges in Translating *in vitro* Bioprinting to *in vivo*

Some of the aforementioned *in situ* hydrogel therapies only rely on the microstructures of hydrogels to facilitate vascularization within the fills, which was unfortunately not effective enough for large-sized constructs. The capability to create precise structures, particularly to establish micro-vascular and interstitial networks within the printed tissues/organs, is crucial to the promotion of effective self-assembly and self-organization of cells, and to the success of bioprinting[14]. Therefore, biofabrication plays an important role in bioprinting not only as cell delivery tools, but also as an effective constructer of optimal micro- and macro-scale architectures. With biofabrication techniques, micro-circulation along with macro-circulation can be potentially created to allow transportation of oxygen, nutrients, and metabolic waste products. It is encouraging to witness the rapid advances in biofabrication, which offer extraordinary opportunities for biotechnology to potentially realize some fascinating advances, e.g. the printing of living tissues/organs. Two excellent review articles on the current states of bioprinting technology have been published by Ozbolat and his colleagues[15,16]. In brief, there are three categories of biofabrication techniques: (i) laser-based; (ii) inkjet-based; and (iii) nozzle-based. These have been concurrently adapted to build *in vitro* bioprinting systems, with the potential to be fused into hybridized systems in the future. It is interesting to reassess each of these technologies with regards to their future *in vivo* applications, particularly their readiness and potential to be handled by surgeons in the operating rooms. Here, the implementation of current bioprinting techniques is briefly reviewed, with comments on some of the techniques to envisage their possible application for *in vivo* bioprinting.

3.1 Bioprinting Techniques

(1) ***Laser-based bioprinting.*** Laser-based bioprinters use laser light to polymerize or solidify biomaterials into fine structures. Laser direct writing (LDW) of cells is a widely used bioprinting approach, in which laser pulses are utilized to selectively transfer cells from a donor container to the building substrate to spatially pattern or construct a cell-jammed structure. A representative of LDW is the laser induced forward transfer (LIFT) bioprinter. The setup of a LIFT-based bioprinter typically comprises of three working units:

a pulsed laser source, a reservoir of cells (donor container), and a building substrate onto which the cells are deposited to for construction as shown in Figure 1[9,17]. It is now feasible to write multiple cell types synchronously with a very high printing resolution at the micron scale[18,19]. The systems that use UV or other light sources are normally defined as 3D projection stereolithography, in which a Digital Micromirror Device (DMD) is applied to selectively project lights onto the photo-curable material to build 3D constructs in a layer-by-layer fashion[20]. Current stereolithography-based *in vitro* bioprinters allow fabrication of 3D bio-constructs with micron- and nano-scale precision, which is helpful in the replication of naturally developed biological structures. The general drawbacks of stereolithography-based approaches include: lower cell viability due to heat generated by the laser or exposure to UV lights; time consuming due to very fine spatial resolutions of the construct; and limited available photocrosslinkable biomaterials. However, in the context of *in vivo* bioprinting, several new challenges for stereolithography-based modalities may also be proposed.

The first challenge regarding *in vivo* laser-based printing may lie in the miniaturization of the devices to allow flexible access to internal organs. For example, the physical dimensions of a Navigator™ laser source are over hundreds of millimeters, which is suitable for a bench-top *in vitro* setup, but it needs to be adapted for *in vivo* application, particularly for biofabrication inside the body. A cable may be used to effectively transmit the laser power generated by bulk sources into the internal defect site, through minimized laser heads with focusing units (e.g., a micro DMD system in the cases of *in vivo* 3D projection-based bioprinting) that are compatible with surgical tools such as endoscopy. An illustration of a cable-transmittable intra-oral teeth printer is shown in Figure 3 as an example of miniaturization of devices. Secondly, low-powered light sources along with effective focusing mechanisms are needed to minimize the exposure of healthy tissues to the laser. For LIFT bioprinters, it would be even more challenging to establish a satisfactory mini "donor slide" over the defect in cases of limited surrounding space. Alternatively, a photosensitive cell-rich resin may be used to fill the defect, following which state-of-the-art laser-based techniques such as two-photon polymerization (2PP) are applied to selectively solidify the resin into desired biomimetic structures with feature sizes of microns.

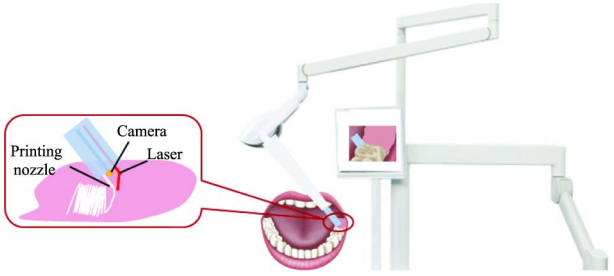

Figure 3. Schematic of laser-based *in vivo* bioprinting in dentistry.

Two-photon polymerization allows for fast construction of structures with submicron (hundreds of nanometers) spatial resolution by using focused femtosecond near-infrared lasers (~800 nm wavelength)[21,22]. The limitation of current 2PP technique in bioprinting practice is that it only allows mono-material resins to be used, which hinders its application in the integral fabrication of heterocellular and multi-material tissues/organs. Other challenges such as stable positioning of light sources (e.g., laser or UV light) and controlling units, along with accurate light focusing in an *in vivo* environment also need to be tackled. In addition, new surgical debridement techniques are needed to allow thorough removal of crosslinkers remnants throughout the *in vivo* bioprinting process without destroying the integration of the newly established construct.

Therefore, while laser-based *in vivo* bioprinting possesses unique advantages such as ultrahigh resolution and precision, it requires advances in biomaterials (e.g. heterocellular and multi-material resins for 2PP stereolithography), engineering and photonics (e.g. development of novel processes which are easy to conduct under extreme *in vivo* conditions), micro-robot- and robot-assisted surgical techniques before it can be moved into clinical practices.

(2) *Inkjet-based bioprinting*. Inkjet-based bioprinters spray bioinks onto the deposition surface, either through drop-on-demand or continuous ejection, to build 3D living constructs. Derived from traditional inkjet printers, this technology has some inherent advantages such as a wide selection of commercially available platforms due to technical sophistication and low cost of device modification. Easy installation of multiple printer heads facilitates heterocellular tissue/organ fabrication and can concurrently achieve a high printing resolution (sub-micron level). Good examples have been demonstrated by a Clemson University-based research group, who modified HP Desktop 550 printers into their own *in vitro* inkjet-

based bioprinting systems. The group successfully fabricated complex cellular patterns and 3D structures of NT2 cells[23], micro channels with human microvascular endothelial cells (HMVEC)[24], and cardiac pseudo tissues with biomaterials as the bio-cartridges[25].

However, the major drawback of inkjet printing lies in the lack of suitable biomaterials which can accommodate the adverse impacts induced by printing through a small orifice. Lack of effective structural integration is another issue related to inkjet-based bioprinting[16]. It is crucial for the printed structures to attain integrity rapidly since the new tissues/organs are expected to survive in the *in vivo* environment immediately after printing, hence for load-bearing tissues, quick establishment of satisfactory mechanical properties through novel biomaterials or/and optimized architectural construction poses a challenge to both biomaterial scientists and engineers that needs to be solved. Due to the size of printing units and their working principle (motors drive printing heads to move on carriage rails), inkjet-based printers seem to be only suitable for *in vivo* repair/fabrication of superficial tissues such as skin. Applying inkjet-based techniques in *in vivo* bioprinting of internal tissues/organs, either through minimization of the entire integral printing unit, or through hybridization of inkjet printing with other methods to form novel working principles is possible will require further improvements in robotics and engineering science.

(3) ***Nozzle-based bioprinting.*** Nozzle- or extrusion-based approaches apply continuous deposition of biomaterials through needles or syringes to construct new tissues/organs. Four major nozzle designs are currently developed for bioprinters of this kind: pressure-actuated, solenoid-actuated, piezoelectric, and volume-actuated nozzles[26]. Nozzle-based bioprinting normally offers a more gentle approach than inkjet bioprinting with regards to cell viability. The most attractive feature of this technique is that multiple cells and biomaterials can be synchronously applied through multiple syringes in a three-dimensional synthesis. In addition, nozzle-based bioprinting seems to be the modality most ready for *in vivo* applications, since arthroscopy-compatible extrusion-based tissue repair has already been clinically applied for decades.

The majority of recently developed bioprinters, or "organ printers", is based on extrusion-based modality due to its intrinsic advantages as mentioned above. Lee *et al*. used a three-axis Cartesian robotic stage to control a four-channel pneumatically driven dispenser, which contains collagen hydrogel precursor, fibroblasts and keratinocytes, in the *in vitro* bioprinting of multi-layered skin substitute[27]. A nozzle-based multi-head bioprinter based on a similar working principle has been developed by Cho and his coworkers, with which 3D open porous structures of decellularized ECM with polycaprolactone (PCL) framework were successfully fabricated[28,29]. To further improve the flexibility and controllability of multi-head bioprinters, a "Multi-arm Bioprinter (MABP)" was developed in 2014 by Ozbolat *et al.*[30]. This nozzle-based printer had two independent arms which allowed concurrent deposition of multiple materials with independently controlled arm motions and material dispensing. A hybrid structure to support the cell spheroids in three dimensions was fabricated using the MABP. It was a brilliant example of how robot-assisted integral fabrication of multiple cells/biomaterials can be done, with great precision in the 3D structures and a vastly accelerated fabrication process.

Possible *in vivo* nozzle-based bioprinting techniques inspired by some modern *in vitro* systems are demonstrated in Figure 4. In addition to similar designs such as co-axial nozzles and dexterous robotic arms that are currently applied in the *in vitro* systems, novel designs with significant microminiaturization features are required to allow maneuverable delivery of bioinks for *in vivo* bioprinting, particularly when access to the internal defect is quite limited. Instead of manually dispensing bioinks at the defective site, microrobots should be used to control the motion of nozzles under the monitor of surgeons to precisely construct biomimetic architectures. In addition, continuous deposition is necessary since this can effectively reduce the fabrication time, and also minimize occurrences of nozzle blockage which is mainly caused by material clogging under static conditions. Compared to other bioprinting modalities covered in this paper, current nozzle-based bioprinting has relatively lower spatial resolutions of the construct[31]. Therefore, developing novel nozzle mechanisms with finer feed control will be of great value for the construction of fine biomimetic structures by nozzle-based bioprinting.

3.2 Bioinks

Though the three aforementioned biofabrication modalities are distinct in their working principles, each have various technical obstacles that need to be overcome, ultimately, success of any bioprinting technologies

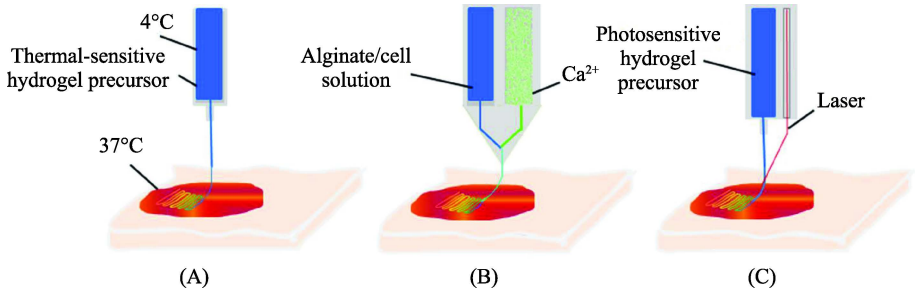

Figure 4. Illustration of components and working principles of *in vivo* nozzle-based bioprinting which are transferrable to the current *in vitro* systems. From the left to right: (A) thermal-sensitive; (B) ion-sensitive; and (C) photo-sensitive *in vivo* gelation.

depend largely on the development of advanced hydrogel systems or building blocks, i.e., cells, biomaterials or their mixtures. Biomaterial has been defined as "…any substance (other than a drug) or combination of substances synthetic or natural in origin, (that) …can be used … as a whole or as a part of a system which treats, augments, or replaces any tissue, organ or function of the body…"[32]. Being shelters to cells, ideal biomaterials should provide cells with comfortable bio-environments and sufficient mechanical/biochemical protection throughout the bioprinting process. The significance of developing a favorable three-dimensional micro-bio-environment for cells in successful tissue regeneration strategies has already been proven both *in vitro* and *in vivo*[33]. To be ideal building blocks, biomaterial components should be highly process-tolerant and printable, with optimal biochemical and mechanical properties which can maintain cellular viability and facilitate tissue fusion and formation. Here, we will briefly review the needs, advances and challenges in cellular and biomaterial science that can contribute to the realization of *in vivo* bioprinting.

(1) *Cell-encapsulating hydrogels for bioprinting.* In native tissues or organs, the ECM is an organized molecular media secreted and regulated by cells. It provides not only sufficient structural and biochemical support to the local cells, but also important mechanical and chemical information for cellular metabolism to mediate cell–cell and cell–matrix interactions. In such a natural environment, nutrients and oxygen are constantly supplied to cells while toxic cellular wastes are continuously removed through extracellular fluids and adjacent capillaries. To date, artificial replication of the fine architectures of multiple-material ECM remains a challenge that lies beyond the capability of all existing micro-biofabrication technologies. However, we can encourage cells to produce their own ECMs by providing a temporary, partially ECM-mimicking but at least highly-hydrated, three-dimensional biomimetic environment, which is the basis of bioprinting. This has been achieved by the development of advanced hydrogels. Hydrogels can assist the transplanted cells in synthesizing natural ECMs through cellular metabolism, and eventually form desired functional tissues through cellular proliferation. Various biologically-relevant hydrogels have been developed for cell encapsulation during the bioprinting process. Based on different gelation mechanisms, the majority of these hydrogels fall into three categories: photo-sensitive, ion-sensitive and thermally-sensitive hydrogels.

Photo-sensitive hydrogels are commonly used in photo-patterning or light-based bioprinting techniques, which involve a photo-polymerization reaction between the hydrogel precursor and photo-initiator to form a user-specific pattern. This kind of hydrogel precursors can be synthesized by modifying natural or synthetic polymers (gelatin and alginate) with methacrylates or acrylates etc.[34–36]. Recent advances indicate that by choosing proper parameters, relatively high viability of the encapsulated cells could be achieved in photo-patterning or bioprinting process[37,38]. More importantly, naturally-derived hydrogels like methacrylated gelatin exhibited comparable biological properties with collagen to support the encapsulated cells' distribution and growth in both *in vitro* and *in vivo* studies[39–41]. However, to apply existing photo-sensitive hydrogels in *in vivo* bioprinting, further optimization should be conducted to significantly shorten the gelatin time as well as developing advanced biocompatible photo-initiators to be safely used in the body.

Alginate is the most used ion-sensitive hydrogel for cell encapsulation in bioprinting[42–46]. The remarkable feature of alginate is its ability to rapidly form hydrogels in a cell-friendly condition upon contact with calcium ions, which makes it a promising hydrogel can-

didate for *in vivo* bioprinting. However, due to the lack of cell-affinity proteins, the ion-crosslinked alginate hydrogel showed limited capability to support the spreading and migration of the encapsulated cells. It is commonly necessary to modify alginate hydrogels with cell-adhesive peptide or mix it with ECM-like components such as gelatin or collagen to improve its biological properties[47–49].

Due to their simple and fully-biocompatible gelation mechanism, thermally-sensitive hydrogels have attracted extensive attentions in bioprinting[27,50]. For example, collagen, as one of the most important components of ECM, remains in its liquid state at 4℃ and form hydrogel at 37℃, which is ideal for *in vivo* bioprinting. A newly developed polypeptide-DNA hydrogel not only showed similar thermoresponsive property as collagen but also possess rapid gelation response. It has been successfully used for *in situ* 3D multi-layer bioprinting[51]. To fully mimic the complex components of native ECM, Pati *et al*. has recently developed a novel thermally-sensitive bioink derived from decellularized matrix, which can be mixed with cells to be used for nozzle-based bioprinting[29]. The printed 3D tissue analogue has shown high cell viability and long-term functions. The continuous innovation in biologically-relevant hydrogel especially the advances in ECM-like, thermally-sensitive hydrogels would accelerate the practice of *in vivo* bioprinting. Novel methods such as the combination of thermo-sensitive properties with chemical crosslinking into a multi-step gelation mechanism may also be helpful to improve the stability of the printed constructs[52].

(2) *Cell aggregates, tissue spheroids and microtissues for bioprinting.* Inspired by developmental biology, cell aggregates or tissue spheroids are intensively studied for their intrinsic capacity to fuse together into a functional construct. By mimicking the tissue fusion phenomenon observed during embryonic development, tissue biofabrication employs delicate positioning of cell aggregates or tissue spheroids to allow them to spontaneously “melt” into macro-tissue constructs[53]. A remarkable property of tissue spheroids is that maximal possible initial cell density can be achieved, which is essential for rapid fluid–solid transition, tissue assembly and maturation to maintain the morphological and compositional integrity of the newly fabricated construct. Kelm *et al*. created living vessel tissues based exclusively on self-assembly (living cellular re-aggregates) of microtissues in an *in vitro* bioreactor in 2010[54]. To provide the fragile tissue spheroids with necessary mechanical supports, rigid internal micro-scaffolds, or macro-porous carriers with micron-sized features were developed[55], and proven to be effective in the cell protection during bioprinting processes. The so-called ‘‘jamming effect’’ was exploited to accelerate the transition from fluid suspensions of spheroids to jammed spheroids solid[56].

For *in vivo* bioprinting, traditional approaches for tissue fusion and maturation are not rapid enough, while the rudimentary constructs currently being built without a prefabricated scaffold generally lack mechanical strength and stiffness. Therefore, new innovative approaches must be explored to achieve rapid fusion of cell aggregates or microtissues, and rapid formation of tissue-like structures with sufficient initial mechanical properties. In addition, a scalable, fast, and safe way to mass produce cell aggregates or microtissues needs to be established to guarantee an ample supply of building blocks for *in vivo* bioprinting. According to a report by Rezende *et al*., scalable biofabrication of tissue spheroids is technically feasible and it is now a subject of ongoing commercialization[57].

Considering the diversity of cell/tissue types, we can hardly find one universal recipe to make bioinks for bioprinting which can repair all tissues/organs. Tissue engineering is a tissue-specific technique per se, in which the specific choice of biomaterial for a specific delivery matrix plays an important role in determining the final properties of the regenerated biological structures. Fortunately, materials engineers have been working closely with cell biologists to improve specifically tailored bioinks for various tissues/organs[58]. These purpose-driven biomaterial researches will provide the essential foundation for the success of organ-specific applications of *in vivo* bioprinting.

4. Prospects for the Future

In the future, when *in vivo* bioprinting technology is ready, the majority of its users will be surgeons. It is therefore important to integrate advanced surgical techniques, such as robot-assisted control systems with user-friendly interfaces, in the commercialization of *in vivo* bioprinting systems. Rigorous requirements on the steadiness of printing units, and the repeatability and consistency of printing construct with microstructures, pose great challenges to surgeons if they are to handle all bioprinting manually in the operating

room. Even the most experienced surgeons cannot match a robot in terms of precision, which is a prerequisite from the perspective of nano- or micro-fabrication. Robot-assisted surgery, which has been labelled as a major step up in precision and surgical quality, seems to be an integral part of *in vivo* bioprinting devices. Currently, a few commercially available robot-assisted surgical systems, such as the MAKOplasty® Knee Replacement System and the da Vinci® Surgical System, have demonstrated their ability to make surgery much easier with perfect accuracy and repeatability achieved.

Another significant trend in surgical technology innovations is minimally invasive surgery. Compared to traditional open surgery, minimally invasive surgery has demonstrated less post-operation pain, less rehabilitation time, and higher quality of life scores[59]. Since patients are better educated and informed thanks to the Internet and television, demand for services such as minimally invasive surgeries is mounting. Back in the early 2000s, Burg and Boland already pointed out the significance of combining injectable biomaterials with tissue-printing technologies in minimally invasive tissue engineering applications[60]. However, most of the currently-developed bioprinting systems require open surgeries that fully expose the organs, due to oversized printing units or intrinsic limitations of their working principles. Therefore, further miniaturizing of device combined with novel working principles for minimally invasive *in vivo* bioprinting would be an interesting direction (Figure 5). To bring bioprinting inside the body, more requirements are imposed on surgical techniques, such as temporary generation of a printing-friendly environment. An example of such surgical techniques can be seen in some arthroscopic cartilage repair surgeries when surgeons use carbon dioxide or normal air insufflation to facilitate the application of the gel under dry conditions[61]. Moreover, advanced real-time imaging or geometric feedback modalities, along with computational modeling and analysis techniques, are of equal importance in the success of *in vivo* bioprinting, for fast and accurate morphological assessment of defects and newly fabricated constructs[62–65].

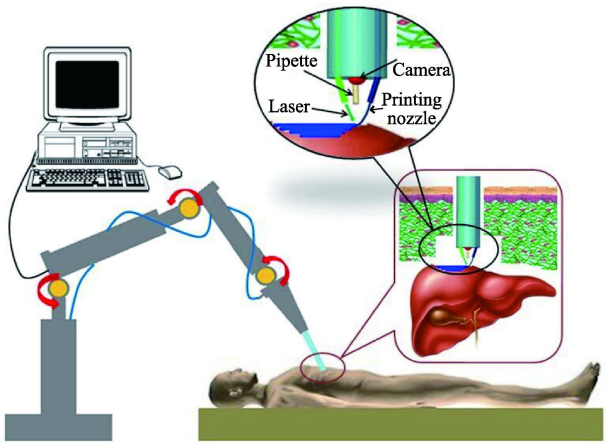

Figure 5. A conceptual vision of a simplified robot-assisted *in vivo* bioprinting system which is compatible with minimally invasive surgeries.

In summary, although the currently prevalent *in vitro* bioprinting strategies are promising in tissue/organ fabrication, particularly for the repair and transplantation of vital organs such as the ones in cardiovascular system, *in vivo* bioprinting will have its own inherent advantages over the *in vitro* systems that include but are not limited to: *in vivo* tissue culture starting from the biofabrication stage to maximize the role of the human body in the regeneration of tissues and organs; real-time, on-site, and precise building of biological constructs to minimize the morphological mismatch between constructs and defects, and to eliminate the influence of physical implantation procedures on the integration of the delicate construct structures; “one stop” fabrication and therapy to be delivered to patients in the operating room, to ultimately reduce operation time as well as risks of contaminations and occurrence of errors due to transportation, manual implantation, etc. To date, although significant advances have occurred since the pilot studies on *in situ* repair of superficial tissues/organs were performed, *in vivo* bioprinting technology is still largely conceptual. As previously mentioned, several core issues such as establishing sufficient vascularization in the newly fabricated tissues/organs, particularly the ones of large sizes, must be addressed synergistically through multidisciplinary development in optimizing bioinks and novel *in vivo* printing systems. With continuous and increasing investments on seeking answers to the global organ shortage crisis, technical advances in cell biology, medicine, materials science, mechatronics, computer science, and robotics have been immensely expedited to benefit interdisciplinary innovations in tissue engineering. It is safe to predict that *in vivo* bioprinting will become realistic in the near future thanks to accelerated growth and sophistication of interdisciplinary knowledge and technologies.

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References

1. Sachs E M, Haggerty J S, Cima M J, *et al.* 1993, Three-dimensional printing techniques, US Patent 5204055 A.
2. Boland T, Xu T, Damon B, *et al.* 2006, Application of inkjet printing to tissue engineering. *Biotechnology Journal*, vol.1(9): 910–917. http://dx.doi.org/10.1002/biot.200600081.
3. Guillotin B, Souquet A, Catros S, *et al.* 2010, Laser assisted bioprinting of engineered tissue with high cell density and microscale organization. *Biomaterials*, vol. 31(28): 7250–7256. http://dx.doi.org/10.1016/j.biomaterials.2010.05.055.
4. Khalil S, Nam J and Sun W, 2005, Multi-nozzle deposition for construction of 3D biopolymer tissue scaffolds. *Rapid Prototyping Journal*, vol.11(1): 9–17. http://dx.doi.org/doi:10.1108/13552540510573347.
5. Wang X, Yan Y and Zhang R, 2009, Recent trends and challenges in complex organ manufacturing. *Tissue Engineering Part B: Reviews*, vol.16(2): 189–197. http://dx.doi.org/10.1089/ten.teb.2009.0576.
6. Binder K W, 2011, *In situ bioprinting of the skin*, thesis, Wake Forest University, viewed WakeSpace database.
7. Binder K W, Zhao W, Aboushwareb T, *et al.* 2010, *In situ* bioprinting of the skin for burns. *Journal of the American College of Surgeons*, vol.211(3): S76. http://dx.doi.org/10.1016/j.jamcollsurg.2010.06.198.
8. Sofokleous P, Stride E, Bonfield W, *et al.* 2013, Design, construction and performance of a portable handheld electrohydrodynamic multi-needle spray gun for biomedical applications. *Materials Science and Engineering: C*, vol.33(1): 213–223. http://dx.doi.org/10.1016/j.msec.2012.08.033.
9. Keriquel V, Guillemot F, Arnault I, *et al.* 2010, *In vivo* bioprinting for computer- and robotic-assisted medical intervention: preliminary study in mice. *Biofabrication*, vol.2(1): 014101. http://dx.doi.org/10.1088/1758-5082/2/1/014101.
10. Cohen D L, Lipton J I, Bonassar L J, *et al.* 2010, Additive manufacturing for *in situ* repair of osteochondral defects. *Biofabrication*, vol.2(3): 035004. http://dx.doi.org/10.1088/1758-5082/2/3/035004.
11. Moreira Teixeira L S, Bijl S, Pully V V, *et al.* 2012, Self-attaching and cell-attracting *in-situ* forming dextran-tyramine conjugates hydrogels for arthroscopic cartilage repair. *Biomaterials*, vol.33(11): 3164–3174. http://dx.doi.org/10.1016/j.biomaterials.2012.01.001.
12. *Biopen to rewrite orthopaedic implants surgery* n.d., viewed May 1, 2015, <http://media.uow.edu.au/news/UOW162803.html>
13. Liang Y, Liu W, Han B, *et al.* 2011, An in situ formed biodegradable hydrogel for reconstruction of the corneal endothelium. *Colloids and Surfaces. B, Biointerfaces*, vol.82(1): 1–7. http://dx.doi.org/10.1016/j.colsurfb.2010.07.043.
14. Xu T, Rodriguez-Devora J I, Reyna-Soriano D, *et al.* 2014, Bioprinting for constructing microvascular systems for organs, in *Rapid Prototyping of Biomaterials: Principles and Applications*, Woodhead Publishing, Cambridge, 201–220.
15. Dababneh A B and Ozbolat I T, 2014, Bioprinting technology: a current state-of-the-art review. *Journal of Manufacturing Science and Engineering*, vol.136(6): 061016. http://dx.doi.org/10.1115/1.4028512.
16. Ozbolat I T and Yu Y, 2013, Bioprinting toward organ fabrication: challenges and future trends. *IEEE Transactions on Bio-medical Engineering*, vol.60(3): 691–699. http://dx.doi.org/10.1109/tbme.2013.2243912.
17. Schiele N R, Chrisey D B and Corr D T, 2011, Gelatin-based laser direct-write technique for the precise spatial patterning of cells. *Tissue Engineering. Part C, Methods*, vol.17(3): 289–298. http://dx.doi.org/10.1089/ten.TEC.2010.0442.
18. Mezel C, Souquet A, Hallo L, *et al.* 2010, Bioprinting by laser-induced forward transfer for tissue engineering applications: jet formation modeling. *Biofabrication*, vol. 2(1): 014103. http://dx.doi.org/10.1088/1758-5082/2/1/014103.
19. Guillemot F, Souquet A, Catros S, *et al.* 2010, High-throughput laser printing of cells and biomaterials for tissue engineering. *Acta Biomaterialia*, vol.6(7): 2494–2500. http://dx.doi.org/10.1016/j.actbio.2009.09.029.
20. Lu Y, Mapili G, Suhali G, *et al.* 2006, A digital micro-mirror device-based system for the microfabrication of complex, spatially patterned tissue engineering scaffolds. *Journal of Biomedical Materials Research. Part A*, vol.77(2): 396–405. http://dx.doi.org/10.1002/jbm.a.30601.
21. Melchels F P W, Feijen J and Grijpma D W, 2010, A review on stereolithography and its applications in bio-

medical engineering. *Biomaterials*, vol.31(24): 6121–6130. http://dx.doi.org/10.1016/j.biomaterials.2010.04.050.

22. Bártolo P, 2011, Stereolithographic Processes, in *Stereolithography*, Springer US, New York, 1–36. http://dx.doi.org/10.1007/978-0-387-92904-0_1.
23. Xu T, Gregory C A, Molnar P, *et al.* 2006, Viability and electrophysiology of neural cell structures generated by the inkjet printing method. *Biomaterials*, vol.27(19): 3580–3588. http://dx.doi.org/10.1016/j.biomaterials.2006.01.048.
24. Cui X and Boland T, 2009, Human microvasculature fabrication using thermal inkjet printing technology. *Biomaterials*, vol.30(31): 6221–6227. http://dx.doi.org/10.1016/j.biomaterials.2009.07.056.
25. Xu T, Baicu C, Aho M, *et al.* 2009, Fabrication and characterization of bio-engineered cardiac pseudo tissues. *Biofabrication*, vol.1(3): 035001. http://dx.doi.org/10.1088/1758-5082/1/3/035001.
26. Billiet T, Vandenhaute M, Schelfhout J, *et al.* 2012, A review of trends and limitations in hydrogel-rapid prototyping for tissue engineering. *Biomaterials*, vol. 33(26): 6020–6041. http://dx.doi.org/10.1016/j.biomaterials.2012.04.050.
27. Lee Y B, Polio S, Lee W, *et al.* 2010, Bio-printing of collagen and VEGF-releasing fibrin gel scaffolds for neural stem cell culture. *Experimental Neurology*, vol. 223(2): 645–652. http://dx.doi.org/10.1016/j.expneurol.2010.02.014.
28. Shim J-H, Lee J-S, Kim J Y, *et al.* 2012, Bioprinting of a mechanically enhanced three-dimensional dual cell- laden construct for osteochondral tissue engineering using a multi-head tissue/organ building system. *Journal of Micromechanics and Microengineering*, vol.22(8): 085014. http://dx.doi.org/10.1088/0960-1317/22/8/085014.
29. Pati F, Jang J, Ha D-H, *et al.* 2014, Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. *Nature Communications*, vol.5. http://dx.doi.org/10.1038/ncomms4935.
30. Ozbolat I T, Chen H and Yu Y, 2014, Development of 'Multi-arm Bioprinter' for hybrid biofabrication of tissue engineering constructs. *Robotics and Computer-Integrated Manufacturing*, vol.30(3): 295–304. http://dx.doi.org/10.1016/j.rcim.2013.10.005.
31. Melchels F P W, Domingos M A N, Klein T J, *et al.* 2012, Additive manufacturing of tissues and organs. *Progress in Polymer Science*, vol.37(8): 1079–1104. http://dx.doi.org/10.1016/j.progpolymsci.2011.11.007.
32. Von Recum A F and Laberge M, 1995, Educational goals for biomaterials science and engineering: Prospective view. *Journal of Applied Biomaterials*, vol.6(2): 137–144. http://dx.doi.org/10.1002/jab.770060209.
33. Huch M, Gehart H, van Boxtel R, *et al.* 2015, Long-term culture of genome-stable bipotent stem cells from adult human liver. *Cell*, vol.160(1–2): 299–312. http://dx.doi.org/10.1016/j.cell.2014.11.050.
34. Nichol J W, Koshy S T, Bae H, *et al.* 2010, Cell-laden microengineered gelatin methacrylate hydrogels. *Biomaterials*, vol.31(21): 5536–5544. http://dx.doi.org/10.1016/j.biomaterials.2010.03.064.
35. Fedorovich N E, Oudshoorn M H, van Geemen D, *et al.* 2009, The effect of photopolymerization on stem cells embedded in hydrogels. *Biomaterials*, vol.30(3): 344–353. http://dx.doi.org/10.1016/j.biomaterials.2008.09.037.
36. Rouillard A D, Berglund C M, Lee J Y, *et al.* 2011, Methods for photocrosslinking alginate hydrogel scaffolds with high cell viability. *Tissue Engineering. Part C, Methods*, vol.17(2): 173–179. http://dx.doi.org/10.1089/ten.TEC.2009.0582.
37. Levett P A, Melchels F P W, Schrobback K, *et al.* 2014, A biomimetic extracellular matrix for cartilage tissue engineering centered on photocurable gelatin, hyaluronic acid and chondroitin sulfate. *Acta Biomaterialia*, vol. 10(1): 214–223. http://dx.doi.org/10.1016/j.actbio.2013.10.005.
38. Ramon-Azcon J, Ahadian S, Obregon R, *et al.* 2012, Gelatin methacrylate as a promising hydrogel for 3D microscale organization and proliferation of dielectrophoretically patterned cells. *Lab on a Chip*, vol.12(16): 2959–2969. http://dx.doi.org/10.1039/C2LC40213K.
39. Nikkhah M, Eshak N, Zorlutuna P, *et al.* 2012, Directed endothelial cell morphogenesis in micropatterned gelatin methacrylate hydrogels. *Biomaterials*, vol.33(35): 9009–9018. http://dx.doi.org/10.1016/j.biomaterials.2012.08.068.
40. Chen Y-C, Lin R-Z, Qi H, *et al.* 2012, Functional human vascular network generated in photocrosslinkable gelatin methacrylate hydrogels. *Advanced Functional Materials*, vol.22(10): 2027–2039. http://dx.doi.org/10.1002/adfm.201101662.
41. Gauvin R, Chen Y C, Lee J W, *et al.* 2012, Microfabrication of complex porous tissue engineering scaffolds using 3D projection stereolithography. *Biomaterials*, vol. 33(15): 3824–3834. http://dx.doi.org/10.1016/j.biomaterials.2012.01.048.
42. Zaborowska M, Sundberg J, Vest N, *et al.* 2010, Alginate bioprinting as a template for 3D bacterial cellulose scaffolds growth for bone healing applications. *Abstracts of Papers of the American Chemical Society*, vol.239.
43. Khalil S and Sun W, 2009, Bioprinting endothelial cells with alginate for 3D tissue Constructs. *Journal of Biomechanical Engineering*, vol.131(11): 111002. http://dx.doi.org/10.1115/1.3128729.
44. Tan Y S E and Yeong W Y, 2014, Direct bioprinting of

alginate-based tubular constructs using multi-nozzle extrusion-based technique. *1st International Conference on Progress in Additive Manufacturing*, http://dx.doi.org/10.3850/978-981-09-0446-3_093.

45. Jia J, Richards D J, Pollard S, *et al.* 2014, Engineering alginate as bioink for bioprinting. *Acta Biomaterialia*, vol.10(10): 4323–4331. http://dx.doi.org/10.1016/j.actbio.2014.06.034.
46. Song S-J, Choi J, Park Y–D, *et al.* 2011, Sodium alginate hydrogel-based bioprinting using a novel multinozzle bioprinting system. *Artificial Organs*, vol.35(11): 1132–1136. http://dx.doi.org/10.1111/j.1525-1594.2011.01377.x.
47. Duan B, Hockaday L A, Kang K H, *et al.* 2013, 3D Bioprinting of heterogeneous aortic valve conduits with alginate/gelatin hydrogels. *Journal of Biomedical Materials Research Part A*, vol.101A(5): 1255–1264. http://dx.doi.org/10.1002/jbm.a.34420.
48. Bidarra S J, Barrias C C, Fonseca K B, *et al.* 2011, Injectable in situ crosslinkable RGD-modified alginate matrix for endothelial cells delivery. *Biomaterials*, vol. 32(31): 7897–7904. http://dx.doi.org/10.1016/j.biomaterials.2011.07.013.
49. Yu J, Du K T, Fang Q, *et al.* 2010, The use of human mesenchymal stem cells encapsulated in RGD modified alginate microspheres in the repair of myocardial infarction in the rat. *Biomaterials*, vol.31(27): 7012–7020. http://dx.doi.org/10.1016/j.biomaterials.2010.05.078.
50. Park J Y, Choi J C, Shim J H, *et al.* 2014, A comparative study on collagen type I and hyaluronic acid dependent cell behavior for osteochondral tissue bioprinting. *Biofabrication*, vol.6(3): 035004. http://dx.doi.org/10.1088/1758-5082/6/3/035004.
51. Li C, Faulkner-Jones A, Dun A R, *et al.* 2015, Rapid formation of a supramolecular polypeptide–DNA hydrogel for *in situ* three-dimensional multilayer bioprinting. *Angewandte Chemie International Edition*, vol. 54(13): 3957–3961. http://dx.doi.org/10.1002/anie.201411383.
52. Wüst S, Godla M E, Müller R, *et al.* 2014, Tunable hydrogel composite with two-step processing in combination with innovative hardware upgrade for cell-based three-dimensional bioprinting. *Acta Biomaterialia*, vol. 10(2): 630–640. http://dx.doi.org/10.1016/j.actbio.2013.10.016.
53. Mironov V, Visconti R P, Kasyanov V, *et al.* 2009, Organ printing: Tissue spheroids as building blocks. *Biomaterials*, vol.30(12): 2164–2174. http://dx.doi.org/10.1016/j.biomaterials.2008.12.084.
54. Kelm J M, Lorber V, Snedeker J G, *et al.* 2010, A novel concept for scaffold-free vessel tissue engineering: Self-assembly of microtissue building blocks. *Journal of Biotechnology*, vol.148(1): 46–55. http://dx.doi.org/10.1016/j.jbiotec.2010.03.002.
55. Liu X, Jin X and Ma P X, 2011, Nanofibrous hollow microspheres self-assembled from star-shaped polymers as injectable cell carriers for knee repair. *Nature Materials*, vol.10(5): 398–406. <http://dx.doi.org/http://www.nature.com/nmat/journal/v10/n5/abs/nmat2999.html#supplementary-information.>
56. Lu P J, Zaccarelli E, Ciulla F, *et al.* 2008, Gelation of particles with short-range attraction. *Nature*, vol. 453(7194): 499–503. <http://dx.doi.org/http://www.nature.com/nature/journal/v453/n7194/suppinfo/nature06931_S1.html.>
57. Rezende R A, Pereira F D A S, Kasyanov V, *et al.* 2013, Scalable Biofabrication of Tissue Spheroids for Organ Printing. *Procedia CIRP*, vol.5(0): 276–281. http://dx.doi.org/10.1016/j.procir.2013.01.054.
58. Ananthanarayanan A, Narmada B C, Mo X, *et al.* 2011, Purpose-driven biomaterials research in liver-tissue engineering. *Trends in Biotechnology*, vol.29(3): 110–118. http://dx.doi.org/10.1016/j.tibtech.2010.10.006.
59. Bragg K, Vanbalen N and Cook N, 2005, Future trends in minimally invasive surgery. *AORN Journal*, vol. 82(6): 1006–1014, 1016–1008; quiz 1019–1022. http://dx.doi.org/10.1016/S0001-2092(06)60252-4.
60. Burg K J L and Boland T, 2003, Minimally invasive tissue engineering composites and cell printing. *Engineering in Medicine and Biology Magazine, IEEE*, vol.22(5): 84–91. http://dx.doi.org/10.1109/MEMB.2003.1256277.
61. Shetty A A, Kim S J, Bilagi P, *et al.* 2013, Autologous collagen-induced chondrogenesis: single-stage arthroscopic cartilage repair technique. *Orthopedics*, vol. 36(5): e648–652. http://dx.doi.org/10.3928/01477447-20130426-30.
62. Cook D, Julias M and Nauman E, 2014, Biological variability in biomechanical engineering research: Significance and meta-analysis of current modeling practices. *Journal of Biomechanics*, vol.47(6): 1241–1250. http://dx.doi.org/10.1016/j.jbiomech.2014.01.040.
63. Hong-Seok P and Chintal S, 2015, Development of high speed and high accuracy 3D dental intra oral scanner. *Procedia Engineering*, vol.100: 1174–1181. http://dx.doi.org/10.1016/j.proeng.2015.01.481.
64. Nam S Y, Ricles L M, Suggs L J, *et al.* 2014, Imaging strategies for tissue engineering applications. *Tissue Engineering Part B: Reviews*, vol.21(1): 88–102. http://dx.doi.org/10.1089/ten.teb.2014.0180.
65. Jin Z, 2014, *Computational Modelling of Biomechanics and Biotribology in the Musculoskeletal System*, Woodhead Publishing, Cambridge, xv–xix. http://dx.doi.org/10.1016/B978-0-85709-661-6.50020-6.