3D bioprinting for tissue engineering: Stem cells in hydrogels

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Abstract: Surgical limitations require alternative methods of repairing and replacing diseased and damaged tissue. Regenerative medicine is a growing area of research with engineered tissues already being used successfully in patients. However, the demand for such tissues greatly outweighs the supply and a fast and accurate method of production is still required.

3D bioprinting offers precision control as well as the ability to incorporate biological cues and cells directly into the material as it is being fabricated. Having precise control over scaffold morphology and chemistry is a significant step towards controlling cellular behaviour, particularly where undifferentiated cells, i.e., stem cells, are used. This level of control in the early stages of tissue development is crucial in building more complex systems that morphologically and functionally mimic *in vivo* tissue.

Here we review 3D printing hydrogel materials for tissue engineering purposes and the incorporation of cells within them. Hydrogels are ideal materials for cell culture. They are structurally similar to native extracellular matrix, have a high nutrient retention capacity, allow cells to migrate and can be formed under mild conditions. The techniques used to produce these materials, as well as their benefits and limitations, are outlined.

Keywords: 3D bioprinting, hydrogels, stem cells, polymers, tissue engineering

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1. Introduction

Whilst 2D printing has had a big influence on everyday living, the advent of additive processing technology in 1986[1] has seen an explosion in innovative ways of producing 3D structures, such as electronic devices[2], aircraft parts[3], medical devices[4] and tissue mimics[5–7]. For clinical applications, early designs based on creating sacrificial moulds as templates for the biomaterials[8] were quickly superseded by aqueous systems that could directly print biological materials[9–11]. Today, the focus is no longer just on providing a suitable platform for cell growth but combining engineering, materials science and cell biology to create a bespoke material of specific dimensions. That material must then integrate well with the patient's healthy tissue and restore functionality to an acceptable level. In the pursuit of developing materials that meet such criteria, manufacturing techniques have also become more complex.

3D bioprinting is the spatial control of the original scaffold preparation techniques with integration of chemical cues and living cells[12]. Printing sensitive biological materials presents new challenges, such as maintaining cell viability throughout the manufactur-

ing process and preventing denaturation of proteins.

In this review we introduce some of the materials used for bioprinting, how stem cells are currently incorporated into the materials and the advantages and limitations of the techniques used to achieve this. Here the focus is to review 3D bioprinting techniques currently employed to create implantable tissue. However, the same techniques may also be employed to create models for studying 3D cell behaviour, diseases and modes of repair.

2. Techniques for 3D Bioprinting

The main approaches to 3D bioprinting are: biomimicry (taking inspiration from nature to develop novel materials), autonomous self-assembly (using cellular organisation to guide the development of bioprinted tissues) and mini-tissue building blocks (identifying and recreating the building blocks of tissues to produce complex systems)[13]. For any one of these strategies, there are a number of techniques that can be employed for their fabrication.

2.1 Inkjet Bioprinting

Based on 2D ink-printing technology, inkjet printing is still the most popular printing method for 3D biological tissues analogues. The first modifications of the technology replaced the ink reservoir with bioink and the paper-feed tray with an *x-y-z* controllable stage[14]. Inkjet printers use thermal or acoustic methods to deliver controlled volumes of the bioink to previously defined locations[15] and build the structure layer-by-layer. Thermal methods generate heat at the print head which forces ink out of the nozzle through pressure pulses. Although temperatures can reach 200–300°C during thermal inkjet printing, this lasts a few microseconds, resulting in an overall temperature rise of 4–10°C for aqueous systems, which has been shown not to have a detrimental effect on cell viability[16]. This method of printing is fast, cheap and readily available. However, although temperature effects on cells has been shown to be minimal, other factors such as print-head clogging, mechanical stress and unreliability in bioink dispensing, present the biggest disadvantages.

Acoustic inkjet printing technology is based on generating pressure in the nozzle by applying a voltage to a piezoelectric crystal which changes the crystal's conformation. Controlling this process precisely allows the bioink to be deposited as droplets[17]. A mod-

ification of this process uses ultrasound to create an acoustic radiation field and form droplets from an air-liquid interface. Control of droplet size and rate of deposition comes from ultrasound pulse, duration and amplitude[18]. The acoustic methods can be modified so that they are not reliant on nozzles[19]. This reduces the risk of clogging and shear stress on cells. There are also no changes in temperature during droplet formation. However, there is a risk of causing cell lysis and membrane damage from the frequencies used to change the piezoelectric crystal shape.

One of the main drawbacks of using either thermal or piezoelectric-based inkjet printing methods is that only liquids with low viscosities are easily printable. This introduces further problems in creating a solid structure once the bioink has been deposited onto the stage[20]. Methods of addressing this issue are outlined in Section 3. Similarly, only low cell numbers can be printed to avoid the nozzle from clogging and to reduce shear stress on the cells[13]. However, once these issues are addressed, inkjet methods offer fast, cheap and high resolution bioprinting with the ability to change drop size and density, thereby the ability to create gradients. When this is coupled with multiple nozzles, it is clear why inkjet printing techniques are so attractive to tissue engineers[21,22].

2.2 Laser-Induced Forward Transfer Bioprinting (LIFT)

Laser-induced forward transfer (LIFT) technology uses pulses of laser focussed on a 'ribbon' upon which the biological material is layered as a solution. The pulse creates a high-pressure bubble which forces the biological material off the ribbon and onto a collector. The technology is not as popular as inkjet and microextrusion for bioprinting but is increasingly being used 23,24. The component set-up for LIFT is entirely different to inkjet and microextrusion technologies and as such the printing resolution and speed is dependent on factors including laser energy, material wettability and surface tension, the spacing between the ribbon and the substrate and material viscosity[25].

The benefits of LIFT are that it is a nozzleless system and so clogging of the print head is no longer an issue, a range of viscosities can be printed without causing a detrimental effect on cell viability[26] and high cell numbers can be printed[27]. These are all advantageous over conventional bioprinting systems.

However, the complexity of LIFT is its biggest downfall. Individual ribbons are required for deposit-

ing different bioinks which can be time-consuming and expensive when printing multiple materials or cell types. Furthermore, the ribbon coating method does not lend itself to distributing cells accurately and metal contaminants are present in the final printed construct; as metal coating is used to create a laser energy absorbing layer on the ribbon.

Even so, as the price for 3D printing is decreasing and LIFT technology is becoming more accessible, several researchers have used it to fabricate clinically relevant constructs, both acellular[28] and cellular [29,30]. As component parts are modified to suit bioprinting for the purpose of tissue engineering, the interest in this technology is likely to grow substantially.

2.3 Microextrusion Bioprinting

Microextrusion printing is one of the most popular and cheapest methods of non-biological printing[31]. The technique uses force to extrude material via a microextrusion head onto a stage, both of which can usually be controlled along the *x*, *y* and *z* axes[32]. For bioprinting, materials can be extruded mechanically or pneumatically[33]. Pneumatic systems are ideal for printing materials that have higher viscosities[34] as they are limited only by the system's air-pressure capabilities and nozzle diameter. The mechanism is simple but delays caused by the compressed gas which controls material flow can affect the printing resolution. Mechanical motor-based microextruders are more complex and provide better spatial resolution but are limited by the forces they can generate and therefore struggle to extrude materials with high viscosities[35].

The temperature of the stage and print head of a microextrusion system can be controlled, which allows a range of materials to be printed[13]. Furthermore, as force is used to extrude the material, high cell densities can be printed, although, as with inkjet methods, the forces generated can affect cell viability. As microextrusion uses higher forces than inkjet printing methods, the cell viability can be as low as 40%[36] or even lower if higher pressures are used. This impact on cell viability can be reduced by lowering the extrusion pressure and printing through nozzles with a large gauge size, although this in turn affects the printing resolution and speed. Nevertheless as microextrusion technology can print high cell densities and can be fitted with multiple extrusion heads, allowing for multi-material or multi-cell printing[37], it remains the most popular method for self-assembly cell printing; through which cells are deposited as spheroids without

a secondary support material[38]. Microextrusion printing has already been used to produce aortic valves[39] and pharmokinetic[40] and disease[41] models. Furthermore, there is room for improvement as the technology is capable of printing non-biological materials at high resolution.

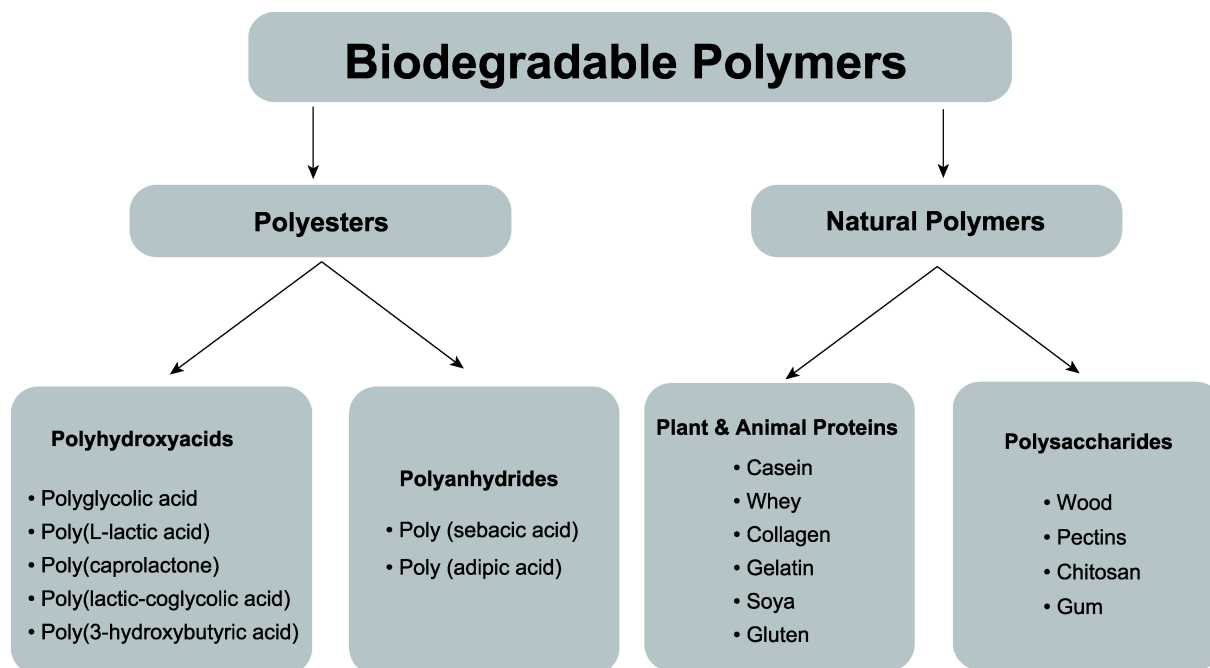### 2.4 Stereolithography and Projection Pattern Bioprinting

Stereolithography is traditionally used to fabricate solid structures from photocurable polymer or resin using a laser and an *x*-*y*-*z*-controlled stage[42]. The technique is based on solid freeform fabrication with polymerised layers printed bottom-up, although top-down stereolithography approaches also exist. The printing resolution is dependent on laser energy and focus. Although traditionally the technique has been used to produce acellular scaffolds, researchers have incorporated photopolymerisable proteins and cell-guiding cues into the scaffolds using stereolithography[43].

Projection stereolithography, also known as digital micromirror device microfabrication, is a modification of the original system which uses micromirrors to create a reflective photomask for fabricating the scaffold layer by layer[44]. Further advancements in the technology have led to the development of a more complex system which allows the entire 3D structure to be polymerised at the same time[45]. Such a system can dramatically reduce the printing speed.

The main drawback with using traditional stereolithography to print scaffolds is that it is not easy to incorporate cells into the structure and maintain viability as it is being fabricated, unless the set-up is modified first[46]. Typically the scaffold is formed first and cells are seeded post-fabrication.

3. Selecting Suitable Materials for 3D Bioprinting

The main challenge in engineering tissues is replicating the *in vivo* environment chemically, mechanically and morphologically. Therefore, the scaffold material on which the cells will be cultured is one of the most important initial choices to be made. The source of these materials may be natural or synthetic (Figure 1). Both types of materials have been used for tissue engineering in equal measure[47–49]. Natural materials are biocompatible while synthetic materials can be modified easily and are therefore easier to handle during

Biodegradable Polymers

Polyesters

Natural Polymers

Polyhydroxyacids

- Polyglycolic acid
- Poly(L-lactic acid)
- Poly(caprolactone)
- Poly(lactic-coglycolic acid)
- Poly(3-hydroxybutyric acid)

Polyanhydrides

- Poly (sebacic acid)
- Poly (adipic acid)

Plant & Animal Proteins

- Casein
- Whey
- Collagen
- Gelatin
- Soya
- Gluten

Polysaccharides

- Wood
- Pectins
- Chitosan
- Gum

Figure 1. Biodegradable polymers used for bioprinting applications.

manufacture. However, natural materials often lack the mechanical integrity required whilst synthetic materials are often not biocompatible[50]. Some researchers have sought to overcome these issues by combining favourable elements from both categories to create hybrid materials[51,52]. Even so, not all of these materials are suited to 3D printing. While the high temperatures and solvents used in the initial 3D printing techniques are not employed for bioprinting, there are still certain criteria, which need to be met when selecting suitable bioprinting materials.

3.1 Printability

It is important to be able to both deposit the material accurately and retain spatial resolution in order to control the overall scaffold geometry. Some bioprinting techniques cannot print viscous materials (such as inkjet methods) while others shear-thin the material and therefore affect its formation (such as microextrusion). Temporal resolution is another aspect which needs to be addressed, as materials that take too long to 'set' will affect the spatial resolution of the scaffold, whilst materials that set too quickly will be in danger of blocking the nozzle. Other factors to consider are whether the cells or biomolecules will encounter shear stress or high temperatures during printing. Current cell-printing technologies report a high variation in cell viabilities; typically between 40% and 90%.

3.2 Biocompatibility

Original expectations of material biocompatibility centered on minimising inflammation and creating materials that would not produce cytotoxic side-effects. Today, however, biocompatibility can include the incorporation of biochemical functionality, i.e. growth factors or growth factor mimics, and nanoscale scaffold morphology to improve and enhance the interaction of cells with the scaffold, and therefore engineered tissue with the *in vivo* environment[13]. It is vital to select a material which can be modified through the printing process such that there is the option of building complexity into the system.

3.3 Degradation

Degradation of a material into smaller chemical units due to material chemistry, oxidising agents, enzymes or ionising radiation and ultrasound occurs via two mechanisms: surface (materials loss layer by layer) or bulk (fragmentation of the whole material)[53]. Figure 2 shows both mechanisms.

The main indicators of degradation are reduction in sample mass, loss of mechanical strength and changes in chemical bonds and groups. Controlled degradation is vital as material loss and a reduction in mechanical integrity of the overall scaffold[54] can alter the cellular response to the material. For example, during bulk
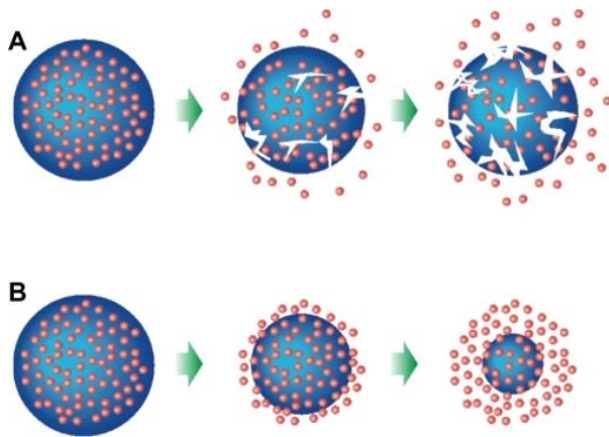
Figure 2. Mechanisms of degradation: bulk (A) and surface (B) degradation.

degradation scaffolds can become more porous, which in turn will have a profound effect on cell migration behaviour and nutrient uptake.

When selecting a bioprintable material which has a suitable degradation profile, it is necessary to also consider whether the cells will contract the scaffold in any way and change its dimensions or whether the material's swelling behaviour will be altered and the effect any by-products from degradation may have on surrounding tissue. A relatively inert and printable material or combination of materials that maintain the correct dimensions could still produce by-products that are toxic or not readily removed by the body[55] and therefore present new challenges *in vivo*.

In order to assess the degradation behaviour of a material, factors such as chemical composition, thermal properties, surface area to volume ratio and stereochemistry must also be taken into consideration.

3.4 Mechanical Strength and Structural Integrity

As stated in Section 2.3, maintaining structural integrity at the same rate as cell growth is highly challenging but necessary. Not only does it provide cells with a physical support, studies have also shown that a mechanical strength which matches *in vivo* conditions can strongly influence cell proliferation and differentiation[56–58]. Several researchers have used a hybrid material approach to create a mechanically suitable environment[59,60]. However, the printability, from changes in viscosities and a mismatch in the most suitable printing technique for the materials, must be assessed and thus further adds to the complexity of the issue. These problems are not insurmountable and several researchers have created 3D printed hybrid scaffolds (see Section 4.3).

4. Using Hydrogels for 3D Bioprinting

Hydrogels are an ideal tissue engineering material which can be sourced naturally, created synthetically or used in combination with other materials[61–64]. Hydrogel networks are comprised of polymer or peptide chains. They have a high content of water, ideal for absorbing high levels of nutrients and oxygen[65], allowing cells to migrate within the scaffold[66] and the waste to diffuse out[67].

Synthesised materials, such as those based on polyethylene glycol and polyacrylamide, offer more control over modification than naturally derived materials such as alginate, collagen, fibrin and hyaluronic acid[62,64].

4.1 Synthetic Materials

Having control over gelation time and mechanical strength are two of the most important elements in hydrogel bioprinting. If the gelation time is too long, the spatial resolution is lost and layers cannot be printed with accuracy. To control the setting time the gelation mechanism can be manipulated by chemically modifying the material, introducing crosslinking agents or varying the polymer content[68]. Müller *et al.*[69] were able to control the printability of Pluronic, a block-copolymer, by mixing acrylated with unmodified Pluronic F127 and stabilising the structure through ultraviolet (UV) crosslinking while Barry *et al.*[70] used direct-write assembly and UV photopolymerisation to produce poly(acrylamide)-based gels for fibroblast culture. With any of the techniques the process of printing a new layer should not disrupt or dissolve the previously deposited material.

Hydrogels as a whole have a high water content which is ideal for maintaining cell viability. However, the material provides low structural support[71]. By using chemical or physical crosslinking methods this can also be improved and therefore solve two major issues using one modification technique. Being able to control scaffold formation in this way would suggest that for bioprinting, synthetic materials, owing to their customisability, are superior to naturally-derived materials. However, cellular interactions and biocompatibility is almost always better on natural materials than synthetic[72]. To improve the biocompatibility of synthetic materials, functional sequences, such as peptide adhesion motifs, can be covalently attached to the material. The drawback of this approach is introducing even more complexity to an already modified system.

4.2 Natural Materials

In contrast, natural materials, although inferior to synthetic hydrogels in terms of controlling gelation kinetics and mechanical strength, are able to chemically and physically mimic native extracellular matrix (ECM). Collagen is the most abundant component of ECM[73]. It is widely used in tissue engineering applications and contains cell-guiding chemical cues, such as the cell adhesion peptide sequence arginine-glycine-aspartic acid (RGD)[74]. However, although it is widely used as a bioprinting material, collagen is an unlikely gold-standard candidate as it contracts and does not retain its original shape.

Hyaluronic acid (HA), is also a naturally derived material which does retain its shape and is already used clinically[75]. HA forms very soft gels but can be modified and crosslinked using a variety of methods including the UV method described in Section 4.1[76] and thiol-modified HA using gold nanoparticles[77] to increase its stiffness. Similarly, fibrin is already used in surgery as a haemostatic agent and sealant[78,79]. The added complexity with fibrin is that it crosslinks through the addition of thrombin. However, it can produce mechanically stable hydrogels and has been blended with other gels for bioprinting purposes[80].

Some natural gels are difficult to print, not because they form soft gels as described in the earlier examples, but because their gelation properties are undesirable. Gelatin is one such material. It forms a gel easily by temperature control but has a melting temperature of 30–35°C[81], which is below the standard physiological temperature of 37°C. Similarly, alginate produces gels easily through cation crosslinking, but unless it is modified with motifs that can guide cells to adhere, proliferate and differentiate, it is relatively inert[82].

4.3 Hybrid Materials

An alternative approach to producing scaffolds with desirable properties is to create a hybrid. A study on methacrylated hyaluronic acid combined with methacrylated gelatin showed that not only could cell viability be maintained but by varying the concentrations of the two materials, the stiffness and viscosity of the hybrid could be controlled[83]. Other researchers have used a similar approach to bioprint scaffolds for a range of uses, including cartilage engineering[84] and to tune material properties for a range of scaffolds[85].

The main issue in using this approach is matching the printable properties of the separate materials or selecting a bioprinting technique which would allow both materials to be printed simultaneously under different conditions. Although the latter adds another level of complexity to printing 3D biocompatible scaffolds, it is a branch of bioprinting that is currently being explored[86].

5. Cell Encapsulation in Hydrogels for Printable Bioinks

The choice of cells for 3D bioprinting is often based on the type of tissue being created. However, as tissues and organs are composed of multiple cell types which have a range of specific functions, it is likely that the bioprinting requirement will be for a mixture of cells. Current methods predominantly involve printing individual cell types in specific patterns, designed to mimic native tissue cell distribution[87]. Although cells have been printed in single drops, with each drop containing one or two cells[88], it is currently not possible to print individual cells reliably. This is not an issue as long as large cell agglomerates (clusters of cells large enough to cause cell death at the centre of the cell mass) can be avoided and cell-to-cell contact can be maintained. The size of these agglomerates will depend on the type of cells used and the ease with which nutrient and waste exchange can occur at the centre of the mass.

For a more efficient system, resembling a native 3D environment, a material-cell composite ink would be more suitable. The ability to encapsulate cells within the material as it is being printed allows researchers to create a more tissue-like environment compared with creating a 2D construct first onto which cells are then seeded[89]. With hydrogels this has been attempted with some success[90], creating cell-laden constructs that contain microvascular networks[91] and are able to integrate well with native tissue[22]. Combining cells with hydrogels is a delicate balance of maintaining high cell viability whilst ensuring that there are not too many cells in the gel to cause hyperplasia or apoptosis, either by optimising the number of cells added at the loading stage of the process or by controlling the rate of cell proliferation post-printing[13].

When using hydrogels with cells, there are a number of factors which could cause cell death. One of the most obvious causes is the method selected for gelation. During crosslinking or temperature-based gelation the cell viability could be substantially affected[92]. The introduction of cytotoxic crosslinking agents

should be avoided and as cells are only able to survive in a narrow temperature range, the list of gel candidates is substantially reduced. However, by combining materials, the list of printable gels could once again be expanded.

Furthermore, the time required for gelation is of importance. The longer it takes for the material to be printed and form the structure, the more likely the layers printed at the start of the process will lose viability, thus limiting the use of the construct. Other factors include the introduction of stress on cells through changes in the viscosity of the gel. While control of viscosity would make the gel more printable, slight changes could lead to low cell viability rates. Stress could also be introduced by methods of extruding the bioink[36] and changes in temperature during the printing process, although the latter is dependent on how long high temperatures are maintained. In their study, Cui *et al.*[16] reported a rise in temperature during printing from 22°C to 46°C. However, as the drops produced cooled within seconds, no significant apoptosis was observed.

6. Using Stem Cell for 3D Bioprinting

The ideal cell type for bioprinting is dependent on the accessibility and availability of the cells, the self-renewal and expansion capacity, differentiation profile and cellular tumorigenicity as well as viability following encapsulation and printing. Stem cells are a particularly attractive cell type as they are pluripotent and able to differentiate into other cell types upon exposure to the correct physical and chemical guidance cues[93]. Within the human body, there are a number of viable sources of stem cells, such as the bone marrow, periosteum and adipose tissue[94–96].

6.1 Stem Cells Selection

Stem cell differentiation can be guided through the incorporation of tissue-specific chemical signals in the scaffold, although some researchers suggest that this may not be necessary to promote differentiation and subsequent tissue regeneration[97]. While the advantages of using pluripotent cells in bioprinting are clear, there are ethical considerations which must be taken into account when using stem cells. Furthermore, the generation of pluripotent stem cells from adult cells (induced pluripotent stem cells, iPSC) pose the risk of tumorigenicity which must also be considered[98]. Ethical issues aside, there are three main categories of stem cells which can be considered for 3D bioprinting:

embryonic, somatic and iPSC.

6.1.1 Embryonic Stem Cells (ESCs)

With the ability to form any cell type and indefinite self-renewal[99], embryonic stem cells (ESCs) are the ideal cell type for tissue engineering. One of the challenges in using ESCs for regenerating or repairing tissue is identifying the conditions needed to drive the cells towards a specific lineage. As cell differentiation is influenced by both chemical and physical cues, the identification of ideal culture conditions adds another level of complexity to an already difficult task.

The biggest drawback of using ESCs is that they are derived from a blastocyst. In some countries, ESCs research is prohibited or severely restricted due to the ethical issues this raises. Furthermore, where research in the field is allowed, the number of cells derived from an embryonic source is low and, unless expanded significantly *in vitro*, is unlikely to meet clinical demand.

6.1.2 Adult Stem Cells

Adult stem cells cover any postnatal somatic cell that is undifferentiated and can self-renew[100]. These cells can be derived from a number of sources including brain, liver and bone marrow[101]. Mesenchymal stem cells (MSCs) are readily available from bone marrow, adipose tissue, amniotic fluid, the synovium and periosteum and are known to be less tumorigenic than their embryonic or fetal counterparts[98]. MSCs are non-haematopoietic, are relatively straightforward to obtain via bone marrow harvesting methods[102] and interact well with a range of materials that may be used for cellular encapsulation to produce viable bioinks. Table 1 features the types of adult MSCs which have been used for bioprinting applications.

Although MSCs can be harvested from the patient's own tissue, and therefore reduce the risk of rejection, only 0.001%–0.01% of total nucleated cells in bone marrow are MSCs[102]. A possible alternative source which could be used is adipose derived MSCs (ADMSCs). Adipose tissue is abundant and many researchers have used ADMSCs successfully towards tissue engineering[94,103,104].

6.1.3 Induced Pluripotent Stem Cells (iPSC)

The discovery that stem cells can be generated directly from adult cells by the introduction of four transcription factors has revolutionised biomedical research[105–108]. By using the patient's own cells, the ethical issues related to stem cell research and the concern surrounding tissue rejection can be avoided. Furthermore, as the iPSCs can be derived from any somatic cell, the

Table 1. Examples of human mesenchymal stem cells used in bioprinting and their performance

No.	Human Cell Type	Scaffold Materials	Regenerated Tissue	Bioprinting Technologies	References
1	Amniotic-derived mesenchymal stem cells	Fibrin-collagen Hydrogel	Skin	Inkjet	Skardal et al. 2012[21]
	(a) Evidence of re-epithelialisation on skin wound in mice with an increase in microvessel density and capillary diameter over 14 days. However, cells did not fully integrate with native tissue.				
2	Adipose-derived mesenchymal stem cells	Alginate	Adipose	Laser-assisted	(a) Gruene et al. 2011[119]
	(a) Adipogenic lineage pathway maintained for 10 days with expression of adipogenic markers similar to those expressed in native adipose tissue.				
3	Bone marrow-derived mesenchymal stem cells	(a) acrylated peptides and acrylated poly(ethylene glycol) (b) poly L-lysine coated carbon nanotubes and acetylated collagen	Bone and Cartilage	Inkjet	(a) Gao et al. 2015[120] (b) Holmes and Zhang 2013[121]
	(a) High cell viability (87.9 ± 5.3%) and good differentiation, evidenced by mineral and cartilage matrix deposition. (b) Biomimetic poly L-lysine coated carbon nanotubes and acetylated collagen can induce proliferation of MSCs.				

yield is high.

However, as a relatively recent discovery, there is still a lot of research to be done on how the cells behave long term. Furthermore, genetic manipulation of cells poses a risk of tumorigenicity[98], introducing new problems in their clinical use. For this reason, some researchers have sought to find alternative routes for generating iPSCs, for example via protein reprogramming[109].

6.2 Stem Cell Bioprinting

When selecting cells for bioprinting, an important factor to consider is the robustness of the cells. Many of the 3D bioprinting technologies outlined in Section 2 can affect cell viability, some of which are discussed in Section 5, and with a limited supply of stem cells, it is essential that this is taken into consideration. While selecting the appropriate bioprinting technique, it is important to ensure that the stem cells retain their pluripotency. If the printing method affects the differentiation potential, primarily through creating a microenvironment to which the stem cells are sensitive[110–112], then a complex scaffold, irrespective of whether it contains cell-guiding functional motifs, is unlikely to produce the desired tissue. Using laser-based printing, Gruene et al.[113] showed that this is possible. Furthermore, early consideration of the interaction between stem cells, the encapsulating material and other cell types used during the bioprinting process could also increase overall viability and help maintain pluripotency[114–116].

As stem cells are sensitive to topography, the scaffold design could strongly influence cell morphology, proliferation and differentiation without the need for additional biological cues[117,118]. Eliminating the addition of growth factors or growth factor-like cues, to reduce bioink complexity, could help improve bioprinting resolution and the overall quality of the product. With the right combination of stem cells, bioprinting technology and scaffold materials, engineering a functional tissue suitable for clinical applications becomes a very real possibility.

7. Future Directions

With the progression in complexity of bioprinted structures, it is clear that the future of clinically relevant 3D printed materials lies in replicating complex and heterogeneous tissues. In this review we have described how technological advancement has occurred in parallel to hybrid material development. Bioprinting is no longer confined to a process for combining one cell type with one material; the emphasis today is to use a variety of material types to create bespoke scaffolds onto which chemical cues can be tethered and multiple cell types can be printed with precision.

Popularity in the use of this technology has led to cheaper systems being made available and therefore more accessible. However, the speed at which the scaffolds are produced is still an area of exploration. This progress is necessary, not only to maintain high cell viability rates but also to scale up the process and fabricate enough scaffolds to meet clinical demands. Kolesky et al.[91] estimate that to print an adult human liver using a single nozzle with a 200 µm diameter, it would take 3 days. However, by switching to a 64-nozzle system under the same conditions this could be reduced to 1 hour. Such a difference in production speeds could result in scaffolds being produced to

meet individual needs quickly whilst reducing the surgical demand for bespoke solutions.

Material compatibility with such advanced systems must also be assessed. A physiologically relevant scaffold must be able to support and guide cell growth and differentiation both chemically and physically. As well as creating complex blends of bioinks, this would require heterogeneous material fabrication and precision-printing to create organized gradients or complex patterns of cells and functional motifs which mimic native ECM more closely. One approach currently being explored to meet these requirements is the use of smart materials, i.e., materials that are able to change their shape, mechanical strength and permeability in response to external or physiological stimuli[117]. Smart hydrogels can respond to changes in pH[122], temperature[123] and electric and magnetic fields[124,125]. These materials are particularly attractive as the scaffold could mould itself as the cells mature.

An alternative approach is to print decellularised ECM directly to provide the structural and chemical cues the cells require. It is believed by some researchers that no matter how complex the hydrogel-based scaffolds become, decellularised ECM is still the closest representation of an *in vivo* environment[126] and therefore the future of bioprinting tissues. The downside with this method is harvesting the ECM first but if tissue-specific ECM can be derived with ease, then this method may help resolve some of the cell functionality issues currently experienced when using other, more conventional scaffolds.

Through the various examples cited in this review, it is clear that bioprinting itself has been successfully used to maintain cell viability and incorporate cell-guiding cues into complex scaffold materials. The main challenge facing researchers in this field today is fine-tuning the technique to mirror native tissue complexity. The goal in tissue engineering is always to improve the patient's quality of life and by creating bespoke materials that are able to regenerate or guide tissue development in a cheap and fast way, 3D bioprinting has become a powerful and highly flexible tool for achieving this. Furthermore, as knowledge on technologies and materials advances, it is entirely plausible that in the future *in situ* bioprinting systems could be developed to both scan the patient's wound site and print the cell-laden scaffold directly into the wound, all without leaving the operating theatre. Significant progress in this area has already been made towards skin[21] and cartilage repair[22].

Author Contributions

Conflict of Interest and Funding

References

1. Hull C W, 1986, Apparatus for production of three-dimensional objects by stereolithography. US 4575330 A, Google Patents.
   http://dx.doi.org/10.1007/s11427-015-4850-3.

2. Leigh S J, Bradley R J, Purssell C P, *et al.*, 2012, A simple, low-cost conductive composite material for 3d printing of electronic sensors. *PLoS ONE*, vol.7: e49365.
   http://dx.doi.org/10.1371/journal.pone.0049365.

3. Berman B, 2012, 3-D printing: The new industrial revolution. *Business Horizons*, vol.55(2): 155–162.
   http://dx.doi.org/10.1016/j.bushor.2011.11.003.

4. Rengier F, Mehndiratta A, von Tengg-Kobligk H, *et al.*, 2010, 3D printing based on imaging data: Review of medical applications. *International Journal of Computer Assisted Radiology and Surgery*, vol.5(4): 335–341.
   http://dx.doi.org/10.1007/s11548-010-0476-x.

5. Mehrban N, Bowen J, Vorndran E, *et al.*, 2013, Structural changes to resorbable calcium phosphate bioceramic aged *in vitro*. *Colloids and Surfaces B: Biointerfaces*, vol.111: 469–478.
   http://dx.doi.org/10.1016/j.colsurfb.2013.06.020.

6. Mehrban N, Paxton J Z, Bowen J, *et al*., 2011, Comparing physichochemical properties of printed and hand cast biocements designed for ligament replacement. *Advances in Applied Ceramics*, vol.110(3): 162–167.
   http://dx.doi.org/10.1179/1743676110Y.0000000012.

7. Ricci J L, Clark E A, Murriky A, *et al*., 2012, Three-dimensional printing of bone repair and replacement materials: Impact on craniofacial surgery. *Journal of Craniofacial Surgery*, vol.23(1): 304–308.
   http://dx.doi.org/10.1097/SCS.0b013e318241dc6e.

8. Tsang V L and Bhatia S N, 2004, Three-dimensional tissue fabrication. *Advanced Drug Delivery Reviews*, vol.56(11): 1635–1647.
   http://dx.doi.org/10.1016/j.addr.2004.05.001.

9. Mironov V, Boland T, Trusk T, *et al*. 2003, Organ printing: Computer-aided jet-based 3D tissue engineering. *Trends in Biotechnology*, vol.21(4): 157–161.

http://dx.doi.org/10.1016/S0167-7799(03)00033-7.

10. Hollister S J, 2005, Porous scaffold design for tissue engineering. *Nature Materials*, vol.4: 518–524.
http://dx.doi.org/10.1038/nmat1421.

11. Seitz H, Rieder W, Irsen S, *et al.*, 2005, Three-dimensional printing of porous ceramic scaffolds for bone tissue engineering. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, vol.74B(2): 782–788.
http://dx.doi.org/10.1002/jbm.b.30291.

12. Khademhosseini A, Langer R, Borenstein J, *et al.*, 2006, Microscale technologies for tissue engineering and biology. *Proceedings of the National Academy of Sciences of the United States of America*, vol.103(8): 2480–2487.
http://dx.doi.org/10.1073/pnas.0507681102.

13. Murphy S V and Atala A, 2014, 3D bioprinting of tissues and organs. *Nature Biotechnology*, vol.32: 773–785.
http://dx.doi.org/10.1038/nbt.2958.

14. Klebe R J, 1988, Cytoscribing: A method for micropositioning cells and the construction of two- and three-dimensional synthetic tissues. *Experimental Cell Research*, vol.179(2): 362–373.
http://dx.doi.org/10.1016/0014-4827(88)90275-3.

15. Singh M, Haverinen H M, Dhagat P, *et al.*, 2010, Inkjet printing — process and its applications. *Advanced Materials*, vol.22(6): 673–685.
http://dx.doi.org/10.1002/adma.200901141.

16. Cui X, Dean D, Ruggeri Z M, *et al.*, 2010, Cell damage evaluation of thermal inkjet printed Chinese hamster ovary cells. *Biotechnology and Bioengineering*, vol.106(6): 963–969.
http://dx.doi.org/10.1002/bit.22762.

17. Tasoglu S and Demirci U, 2013, Bioprinting for stem cell research. *Trends in Biotechnology*, vol.31(1): 10–19.
http://dx.doi.org/10.1016/j.tibtech.2012.10.005.

18. Xu C, Zhang M, Huang Y, *et al.*, 2014, Study of droplet formation process during drop-on-demand inkjetting of living cell-laden bioink. *Langmuir*, vol.30(30): 9130–9138.
http://dx.doi.org/10.1021/la501430x.

19. Demirci U and Montesano G, 2007, Single cell epitaxy by acoustic picolitre droplets. *Lab on a Chip*, vol.7(9): 1139–1145.
http://dx.doi.org/10.1039/B704965J.

20. Derby B, 2012, Printing and prototyping of tissues and scaffolds. *Science*, vol.338(6109): 921–926.
http://dx.doi.org/10.1126/science.1226340.

21. Skardal A, Mack D, Kapetanovic E, *et al.*, 2012, Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds. *Stem Cells Translational Medicine*, vol.1(11): 792–802.
http://dx.doi.org/10.5966/sctm.2012-0088.

22. Cui X, Breitenkamp K, Finn MG, *et al.*, 2012, Direct human cartilage repair using three-dimensional bioprinting technology. *Tissue Engineering: Part A*, vol.18(11–12): 1304–1312.
http://dx.doi.org/10.1089/ten.TEA.2011.0543.

23. Duocastella M, Colina M, Fernández-Pradas J M, *et al.*, 2007, Study of the laser-induced forward transfer of liquids for laser bioprinting. *Applied Surface Science*, vol.253(19): 7855–7859.
http://dx.doi.org/10.1016/j.apsusc.2007.02.097.

24. Mézel 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.

25. Ali M, Pages E, Ducom A, *et al.*, 2014, Controlling laser-induced jet formation for bioprinting mesenchymal stem cells with high viability and high resolution. *Biofabrication*, vol.6(4): 045001.
http://dx.doi.org/10.1088/1758-5082/6/4/045001.

26. Hopp B, Smausz T, Krez N, *et al.*, 2005, Survival and proliferative ability of various living cell types after laser-induced forward transfer. *Tissue Engineering*, vol.11(11–12): 1817–1823.
http://dx.doi.org/10.1089/ten.2005.11.1817.

27. 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.

28. Zopf D A, Hollister S J and Nelson M E, 2013, Bioresorbable airway splint created with a three-dimensional printer. *The New England Journal of Medicine*, vol.368: 2043–2045.
http://dx.doi.org/10.1056/NEJMc1206319.

29. Michael S, Sorg H, Peck C-T, *et al.*, 2013, Tissue engineered skin substitutes created by laser-assisted bioprinting form skin-like structures in the dorsal skin fold chamber in mice. *PLoS ONE*, vol.8(3): e57741.
http://dx.doi.org/10.1371/journal.pone.0057741.

30. 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.

31. Ragaert K, Cardon L, Dekeyser A, *et al.*, 2010, Machine design and processing considerations for the 3D plotting of thermoplastic scaffolds. *Biofabrication*, vol.2(1): 014107.
http://dx.doi.org/10.1088/1758-5082/2/1/014107.

32. Zhang X and Zhang Y, 2015, Tissue engineering applications of three-dimensional bioprinting. *Cell Biochemistry and Biophysics*, vol.72(3): 777–782.
http://dx.doi.org/10.1007/s12013-015-0531-x.

33. Malda J, Visser J, Melchels F P, *et al.*, 2013, 25th anniversary article: Engineering hydrogels for Biofabrica-

tion. *Advanced Materials*, vol.25(36): 5011–5028.
http://dx.doi.org/10.1002/adma.201302042.

34. Tirella A, Vozzi F, Vozzi G, *et al.*, 2011, PAM2 (Piston Assisted Microsyringe): A new rapid prototyping technique for biofabrication of cell incorporated scaffolds. *Tissue Engineering: Part C*, vol. 17(2): 229–237.
http://dx.doi.org/10.1089/ten.tec.2010.0195.

35. DeSimone E, Schacht K, Jungst T, *et al.*, 2015, Biofabrication of 3D constructs: Fabrication technologies and spider silk proteins as bioinks. *Pure and Applied Chemistry*, vol.87(8): 737–749.
http://dx.doi.org/10.1515/pac-2015-0106.

36. Chang R, Nam J and Sun W, 2008, Effects of dispensing pressure and nozzle diameter on cell survival from solid freeform fabrication–based direct cell writing. *Tissue Engineering: Part A*, vol.14(1): 41–48.
http://dx.doi.org/10.1089/ten.a.2007.0004.

37. 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/10.1108/13552540510573347.

38. Jakab K, Norotte C, Marga F, *et al.*, 2010, Tissue engineering by self-assembly and bio-printing of living cells. *Biofabrication*, vol.2(2): 022001.
http://dx.doi.org/10.1088/1758-5082/2/2/022001.

39. 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 A,* vol.101A(5): 1255–1264.
http://dx.doi.org/10.1002/jbm.a.34420.

40. Chang R, Nam J and Sun W, 2008, Direct cell writing of 3D microorgan for *in vitro* pharmacokinetic model. *Tissue Engineering Part C: Methods*, vol.14(2): 157–166.
http://dx.doi.org/10.1089/ten.tec.2007.0392.

41. Xu F, Celli J, Rizvi I, *et al.*, 2011, A three-dimensional in vitro ovarian cancer coculture model using a high-throughput cell patterning platform. *Biotechnology Journal*, vol.6(2): 204–212.
http://dx.doi.org/10.1002/biot.201000340.

42. Melchels F P W, Feijen J and Grijpma D W, 2010, A review on stereolithography and its applications in biomedical engineering. *Biomaterials*, vol.31(24): 6121–6130.
http://dx.doi.org/10.1016/j.biomaterials.2010.04.050.

43. Castro N J, O'Brien J and Zhang LG, 2015, Integrating biologically inspired nanomaterials and table-top stereolithography for 3D printed biomimetic osteochondral scaffolds. *Nanoscale*, vol.7(33): 14010.
http://dx.doi.org/10.1039/c5nr03425f.

44. Grogan S P, Chung P H, Soman P, *et al.*, 2013, Digital micromirror device projection printing system for meniscus tissue engineering. *Acta Biomaterialia*, vol.9(7): 7218–7226.
http://dx.doi.org/10.1016/j.actbio.2013.03.020.

45. Guo M, Qu X, Zhu W, *et al.*, 2014, Bio-inspired detoxification using 3D-printed hydrogel nanocomposites. *Nature Communications*, vol.5: 3774.
http://dx.doi.org/10.1038/ncomms4774.

46. Dhariwala B, Hunt E and Boland T, 2014, Rapid prototyping of tissue-engineering constructs, using photopolymerisable hydrogels and stereolithography. *Tissue Engineering*, vol.10(9–10): 1316–1322.
http://dx.doi.org/10.1089/ten.2004.10.1316.

47. Lee K Y and Mooney D J, 2001, Hydrogels for tissue engineering. *Chemical Reviews*, vol.101(7): 1869–1879.
http://dx.doi.org/10.1021/cr000108x.

48. Hutmacher D W, Goh J C and Teoh S H, 2001, An introduction to biodegradable materials for tissue engineering applications. *Annals of the Academy of Medicine, Singapore*, vol.30: 183–191.

49. Drury J L, Mooney D J and 2003, Hydrogels for tissue engineering: Scaffold design variables and applications. *Biomaterials*, vol.24(24): 4337–4351.
http://dx.doi.org/10.1016/S0142-9612(03)00340-5.

50. O'Brien F J, 2011, Biomaterials & scaffolds for tissue engineering. *Materials Today*, vol.14(3): 88–95.
http://dx.doi.org/10.1016/S1369-7021(11)70058-X.

51. Iwasaki N, Yamane S T, Majima T, *et al.*, 2004, Feasibility of polysaccharide hybrid materials for scaffolds in cartilage tissue engineering: evaluation of chondrocyte adhesion to polyion complex fibers prepared from alginate and chitosan. *Biomacromolecules*, vol.5(3): 828–833.
http://dx.doi.org/10.1021/bm0400067.

52. Costa V C, Costa H S, Vasconcelos W L, *et al.*, 2007, Preparation of hybrid biomaterials for bone tissue engineering. *Materials Research*, vol.10(1): 21–26.
http://dx.doi.org/10.1590/S1516-14392007000100006.

53. Shah A A, Hasan F, Hameed A, *et al*., 2008, Biological degradation of plastics: a comprehensive review. *Biotechnology Advances*, vol.26(3): 246–265.
http://dx.doi.org/10.1016/j.biotechadv.2007.12.005.

54. Göpferich A, 1996, Mechanisms of polymer degradation and erosion. *Biomaterials*, vol.17(2): 103–114.
http://dx.doi.org/10.1016/0142-9612(96)85755-3.

55. Salacinski H J, Tai N R, Carson R J, *et al.*, 2002, *In vitro* stability of a novel compliant poly(carbonate-urea) urethane to oxidative and hydrolytic stress. *Journal of Biomedical Materials Research*, vol.59(2): 207–218.
http://dx.doi.org/10.1002/jbm.1234.

56. Steven M M and George G H, 2005, Exploring and engineering the cell surface interface. *Science*, vol.310(5751): 1135–1138.
http://dx.doi.org/10.1126/science.1106587.

57. Seidlits S K, Khaing Z Z, Petersen R R, *et al.*, 2010, The effects of hyaluronic acid hydrogels with tunable mechanical properties on neural progenitor cell differentiation. *Biomaterials*, vol.31(14): 3930–3940.
http://dx.doi.org/10.1016/j.biomaterials.2010.01.125.

58. Murphy C M, Matsiko A, Haugh M G, *et al.*, 2012, Mesenchymal stem cell fate is regulated by the composition and mechanical properties of collagen-glycosaminoglycan scaffolds. *Journal of the Mechanical Behaviour of Biomedical Materials*, vol.11: 53–62.
http://dx.doi.org/10.1016/j.jmbbm.2011.11.009.

59. Shim J H, Kim J Y, Park M, *et al.*, 2011, Development of a hybrid scaffold with synthetic biomaterials and hydrogel using solid freeform fabrication technology. *Biofabrication*, vol.3(3): 034102.
http://dx.doi.org/10.1088/1758-5082/3/3/034102.

60. Ahn S H, Kim Y B, Lee H J, *et al.*, 2012, A new hybrid scaffold constructed of solid freeform-fabricated PCL struts and collagen struts for bone tissue regeneration: fabrication, mechanical properties, and cellular activity. *Journal of Materials Chemistry*, vol.22(31): 15901–15909.
http://dx.doi.org/10.1039/C2JM33310D.

61. Tan H and Marra K G, 2010, Injectable, biodegradable hydrogels for tissue engineering applications. *Materials*, vol.3(3): 1746–1767.
http://dx.doi.org/10.3390/ma3031746.

62. Zhu J and Marchant R E, 2011, Design properties of hydrogel tissue-engineering scaffolds. *Expert Review of Medical Devices*, vol.8(5): 607–626.
http://dx.doi.org/10.1586/erd.11.27.

63. Hunt JA, Chen R, van Veen T, *et al.*, 2014, Hydrogels for tissue engineering and regenerative medicine. *Journal of Materials Chemistry B*, vol.2(33): 5319–5338.
http://dx.doi.org/10.1039/C4TB00775A.

64. Ahmed E M, 2015, Hydrogel: Preparation, characterization, and applications: A review. *Journal of Advanced Research*, vol.6(2): 105–121.
http://dx.doi.org/10.1016/j.jare.2013.07.006.

65. Skardal A and Atal A 2015, Biomaterials for integration with 3-D bioprinting. *Annals of Biomedical Engineering*, vol.43(3): 730–746.
http://dx.doi.org/10.1007/s10439-014-1207-1.

66. Vu LT, Jain G, Veres B D, *et al.*, 2015, Cell migration on planar and three-dimensional matrices: A hydrogel-based perspective. *Tissue Engineering Part B: Reviews*, vol.21(1): 67–74.
http://dx.doi.org/10.1089/ten.TEB.2013.0782.

67. Amsden B, 1998, Solute diffusion within hydrogels. Mechanisms and models. *Macromolecules*, vol.31(23): 8382–8395.
http://dx.doi.org/10.1021/ma980765f.

68. Tan H, Li H, Rubin J P, *et al.*, 2011, Controlled gelation and degradation rates of injectable hyaluronic acid-based hydrogels through a double crosslinking strategy. *Journal of Tissue Engineering and Regenerative Medicine*, vol.5(10): 790–797.
http://dx.doi.org/10.1002/term.378.

69. Müller M, Becher J, Schnabelrauch M, *et al.*, 2015, Nanostructured Pluronic hydrogels as bioinks for 3D bioprinting. *Biofabrication*, vol.7(3): 035006.
http://dx.doi.org/10.1088/1758-5090/7/3/035006.

70. Barry RA, Shepherd R F, Hanson J N, *et al.*, 2009, Direct-write assembly of 3D hydrogel scaffolds for guided cell growth. *Advanced Materials*, vol.21(23): 2407–2410.
http://dx.doi.org/10.1002/adma.200803702.

71. Cha C, Soman P, Zhu W, *et al.*, 2014, Structural reinforcement of cell-laden hydrogels with microfabricated three dimensional scaffolds. *Biomaterials Science*, vol.2(5): 703–709.
http://dx.doi.org/10.1039/C3BM60210A.

72. Geckil H, Xu F, Zhang X, *et al.*, 2010, Engineering hydrogels as extracellular matrix mimics. *Nanomedicine (London),* vol.5(3): 469–484.
http://dx.doi.org/10.2217/nnm.10.12.

73. Frantz C, Stewart K M and Weaver V M, 2010, The extracellular matrix at a glance. *Journal of Cell Science*, vol.123: 4195–4200.
http://dx.doi.org/10.1242/jcs.023820.

74. Taubenberger A V, Woodruff M A, Bai H, *et al.*, 2010, The effect of unlocking RGD-motifs in collagen I on pre-osteoblast adhesion and differentiation. *Biomaterials*, vol.31(10): 2827–2835.
http://dx.doi.org/10.1016/j.biomaterials.2009.12.051.

75. Galus R, Antiszko A and Wlodarski P, 2006, Clinical applications of hyaluronic acid. Polski Merkuriusz Lekarski, vol.20(119): 606–608.

76. Skardal A, Zhang J, McCoard L, *et al.*, 2010, Photocrosslinkable hyaluronan-gelatin hydrogels for two-step bioprinting. *Tissue Engineering: Part A*, vol.16(8): 2675–2685.
http://dx.doi.org/10.1089/ten.TEA.2009.0798.

77. Skardal A, Zhang J, McCoard L, *et al*., 2010, Dynamically crosslinked gold nanoparticle-hyaluronan hydrogels. *Advanced Materials*, vol.22(42): 4736–4740.
http://dx.doi.org/10.1002/adma.201001436.

78. Jackson M R, 2001, Fibrin sealants in surgical practice: An overview. *The American Journal of Surgery*, vol.182(2): S1–S7.
http://dx.doi.org/10.1016/S0002-9610(01)00770-X.

79. Traver M A and Assimos DG, 2006, New generation tissue sealants and hemostatic agents: Innovative urologic applications. *Reviews in Urology*, vol.8(3): 104–111.

80. Ahmed T A, Dare EV and Hincke M, 2008, Fibrin: a versatile scaffold for tissue engineering applications. *Tissue Engineering Part B: Reviews*, vol.14(2): 199–215.
http://dx.doi.org/10.1089/ten.teb.2007.0435.

81. Masutani E M, Kinoshita C K, Tanaka T T, *et al*., 2014, Increasing thermal stability of gelatin by UV-induced cross-linking with glucose. *International Journal of Biomaterials*, vol.2014: Article ID 979636.
http://dx.doi.org/10.1155/2014/979636.

82. Sun J and Tan H, 2013, Alginate-based biomaterials for

regenerative medicine applications. *Materials*, vol.6(4): 1285–1309.
http://dx.doi.org/10.3390/ma6041285.

83. Duan B, Kapetanovic E, Hockaday L A, *et al.*, 2014, Three-dimensional printed trileaflet valve conduits using biological hydrogels and human valve interstitial cells. *Acta Biomaterialia*, vol.10(5): 1836–1846.
http://dx.doi.org/10.1016/j.actbio.2013.12.005.

84. Kundu J, Shim J-H, Jang J, *et al.*, 2013, An Additive manufacturing-based PCL-alginate-chondrocyte bioprinted scaffold for cartilage tissue engineering. *Journal of Tissue Engineering and Regenerative Medicine,* vol.9(11): 1286–1297.
http://dx.doi.org/10.1002/term.1682.

85. Rutz A L, Hyland K E, Jakus A E, *et al.*, 2015, A multi-material bioink method for 3D printing tunable, cell-compatible hydrogels. *Advanced Materials*, vol.27(9): 1607–1614.
http://dx.doi.org/10.1002/adma.201405076.

86. Ozbolat I T, Chen H and Yu Y, 2014, Development of 'multi-arm bioprinter' for hybrid biofabrication of tissue engineered constructs. *Robotics and Computer-Integrated Manufacturing*, vol.30(3): 295–304.
http://dx.doi.org/10.1016/j.rcim.2013.10.005.

87. Khalil S and Sun W, 2009, Bioprinting endothelial cells with alginate for 3D tissue constructs. *Journal of Biomedical Engineering*, vol.131(11): 111002.
http://dx.doi.org/10.1115/1.3128729.

88. Nakamura M, Iwanaga S, Henmi C, *et al.*, 2010, Biomatrices and biomaterials for future developments of bioprinting and biofabrication. *Biofabrication*, vol.2(1): 014110.
http://dx.doi.org/10.1088/1758-5082/2/1/014110.

89. Pati F, Jang J, Ha D-H, *et al.*, 2013, Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. *Nature Communications*, vol.5: Article number 3935.
http://dx.doi.org/10.1038/ncomms4935.

90. Xu T, Jin J, Gregory C, *et al.*, 2005, Inkjet printing of viable mammalian cells. *Biomaterials*, vol.26(1): 93–99.
http://dx.doi.org/10.1016/j.biomaterials.2004.04.011.

91. Kolesky D B, Truby R L, Gladman A S, *et al.*, 2014, 3D bioprinting of vascularized, heterogenous cell-laden tissue constructs. *Advanced Materials*, vol.26(19): 3124–3130.
http://dx.doi.org/10.1002/adma.201305506.

92. Williams C G, Malik A N, Kim T K, *et al.*, 2005, Variable cytocompatibility of six cell lines with photoinitiators used for polymerizing hydrogels and cell encapsulation. *Biomaterials*, vol. 26(11): 1211–1218.
http://dx.doi.org/10.1016/j.biomaterials.2004.04.024.

93. Bianco P and Robey P G, 2001, Stem cells in tissue engineering. *Nature*, vol.414: 118–121.
http://dx.doi.org/10.1038/35102181.

94. Gimble J M and Guilak F, 2003, Adipose-derived adult stem cells: Isolation, characterization, and differentiation potential. *Cytotherapy*, vol.5(5): 362–369.
http://dx.doi.org/10.1080/14653240310003026.

95. Winter A, Breit S, Parsch D, *et al.*, 2003, Cartilage-like gene expression in differentiated human stem cell spheroids: A comparison of bone marrow–derived and adipose tissue–derived stromal cells. *Arthritis and Rheumatology*, vol.48(2): 418–429.
http://dx.doi.org/10.1002/art.10767.

96. Barry F P and Murphy J M, 2004, Mesenchymal stem cells: Clinical applications and biological characterization. *The International Journal of Biochemistry and Cell Biology*, vol.36(4): 568–584.
http://dx.doi.org/10.1016/j.biocel.2003.11.001.

97. Dalby M J, Riehle M O, Johnstone H, *et al.*, 2002, *In vitro* reaction of endothelial cells to polymer demixed nanotopography. *Biomaterials*, vol.23(14): 2945–2954.
http://dx.doi.org/10.1016/S0142-9612(01)00424-0.

98. Knoepfler P S, 2009, Deconstructing stem cell tumorigenicity: A roadmap to safe regenerative medicine. *Stem cells*, vol.27(5): 1050–1056.
http://dx.doi.org/10.1002/stem.37.

99. Vats A, Tolley N S, Bishop A E, *et al.*, 2005, Embryonic stem cells and tissue engineering: delivering stem cells to the clinic. *Journal of the Royal Society of Medicine*, vol.98(8): 346–350.
http://dx.doi.org/10.1258/jrsm.98.8.346.

100. Tsai C C, Su P F, Huang Y F, *et al.*, 2012, Oct4 and Nanog directly regulate Dnmt1 to maintain self-renewal and undifferentiated state in mesenchymal stem cells. *Molecular Cell*, vol.47(2): 169–182
http://dx.doi.org/10.1016/j.molcel.2012.06.020.

101. Körbling M, Estrov Z and Champlin R, 2003, Adult stem cells and tissue repair. *Bone Marrow Transplantation*, vol.32: S23–S24.
http://dx.doi.org/10.1038/sj.bmt.1703939.

102. Pittenger M F, Mackay A M, Beck S C, *et al.*, 1999, Multilineage potential of adult human mesenchymal stem cells. *Science*, vol.284: 143–147.
http://dx.doi.org/10.1126/science.284.5411.143.

103. Sterodimas A, de Faria J Nicaretta B, *et al.*, 2010, Tissue engineering with adipose-derived stem cells (ADSCs): Current and future applications. *Journal of Plastic, Reconstructive & Aesthetic Surgery*, vol.63(11): 1886–1892.
http://dx.doi.org/10.1016/j.bjps.2009.10.028.

104. Zuk P 2013, Adipose-derived stem cells in tissue regeneration: A review. *International Scholarly Research Notices*, vol.2013: Article ID 713959.
http://dx.doi.org/10.1155/2013/713959.

105. Takahashi K and Yamanaka S, 2006, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, vol.126(4): 663–676.

http://dx.doi.org/10.1016/j.cell.2006.07.024.

106. Wang A, Tang Z, Park I-H, *et al.*, 2011, Induced Pluripotent Stem Cells for Neural Tissue Engineering. *Biomaterials*, vol.32(22): 5023–502.
http://dx.doi.org/10.1016/j.biomaterials.2011.03.070.

107. Diekman B O, Christoforou N, Willard V P, *et al.*, 2012, Cartilage tissue engineering using differentiated and purified induced pluripotent stem cells. *Proceedings of the National Academy of Sciences of the United States of America*, vol.109: 19172–19177.
http://dx.doi.org/10.1073/pnas.1210422109.

108. De Peppo G M, Marcos-Campos I, Kahler D J, *et al.*, Engineering bone tissue substitutes from human induced pluripotent stem cells. *Proceedings of the National Academy of Sciences of the United States of America*, vol.110: 8680–8685.
http://dx.doi.org/10.1073/pnas.1301190110.

109. Cho H-J, Lee C-S, Kwon Y-W, *et al.*, 2010, Induction of pluripotent stem cells from adult somatic cells by protein-based reprogramming without genetic manipulation. *Blood*, vol.116(3): 386–395.
http://dx.doi.org/10.1182/blood-2010-02-269589.

110. Peerani R, Rao B M, Bauwens C, *et al.*, 2007, Niche-mediated control of human embryonic stem cell self-renewal and differentiation. *The EMBO Journal*, vol.26: 4744–4755.
http://dx.doi.org/10.1038/sj.emboj.7601896.

111. Park J, Cho C H, Parashurama N, *et al.*, 2007, Microfabrication-based modulation of embryonic stem cell differentiation. *Lab on a Chip*, vol.7(8):1018–1028.
http://dx.doi.org/10.1039/B704739H.

112. Raof N A, Schiele N R, Xie Y, *et al.*, 2011, The maintenance of pluripotency following laser direct-write of mouse embryonic stem cells. *Biomaterials*, vol.32(7): 1802–1808.
http://dx.doi.org/10.1016/j.biomaterials.2010.11.015.

113. Gruene M, Deiwick A, Koch L, *et al.*, 2011, Laser printing of stem cells for biofabrication of scaffold-free autologous grafts. *Tissue Engineering Part C: Methods*, vol.17(1): 79–87.
http://dx.doi.org/10.1089/ten.tec.2010.0359.

114. Le Visage C, Dunham B, Flint P, *et al.*, 2004, Coculture of mesenchymal stem cells and respiratory epithelial cells to engineer a human composite respiratory mucosa. *Tissue Engineering*, vol.10(9–10): 1426–1435.
http://dx.doi.org/10.1089/ten.2004.10.1426.

115. Gaebel R, Ma N, Liu J, *et al.*, 2011, Patterning human stem cells and endothelial cells with laser printing for cardiac regeneration. *Biomaterials*, vol.32(35): 9218–9230.
http://dx.doi.org/10.1016/j.biomaterials.2011.08.071.

116. Teoh G Z, Crowley C, Birchall M A, *et al.*, 2015, Development of resorbable nanocomposite tracheal and bronchial scaffolds for paediatric applications. *British Journal of Surgery*, vol.102(2): e140–e150.
http://dx.doi.org/10.1002/bjs.9700.

117. Wang S, Lee J M, and Yeong W Y, 2015, Smart hydrogels for 3D bioprinting. *International Journal of Bioprinting*, vol.1: 3–14.
http://dx.doi.org/10.18063/IJB.2015.01.005.

118. Seras-Franzoso J, Tsimbouri P M, Burgess K V, *et al.*, 2014, Topographically targeted osteogenesis of mesenchymal stem cells stimulated by inclusion bodies attached to polycaprolactone surfaces. *Nanomedicine*, vol.9(2): 207–220.
http://dx.doi.org/10.2217/nnm.13.43.

119. Gruene M, Pflaum M, Deiwick A, *et al.*, 2011 Adipogenic differentiation of laser-printed 3D tissue grafts consisting of human adipose-derived stem cells. *Biofabrication*, vol.3(1): 015005.
http://dx.doi.org/10.1088/1758-5082/3/1/015005.

120. Gao G, Yonezawa T, Hubbell K, *et al.*, 2015, Inkjet-bioprinted acrylated peptides and PEG hydrogel with human mesenchymal stem cells promote robust bone and cartilage formation with minimal printhead clogging. *Biotechnology Journal*, vol.10(10): 1568–1577.
http://dx.doi.org/10.1002/biot.201400635.

121. Holmes B and Zhang L G, 2013, Enhanced human bone marrow mesenchymal stem cell functions in 3D bioprinted biologically inspired osteochondral construct. *The American Society of Chemical Engineers Proceedings*, vol.3A: V03AT03A002.
http://dx.doi.org/10.1115/IMECE2013-66118.

122. Lee V, Singh G, Tarasatti J P, *et al.*, 2014, Design and fabrication of human skin by three-dimensional bioprinting. *Tissue Engineering Part C: Methods*, vol.20(6): 473–484.
http://dx.doi.org/10.1089/ten.TEC.2013.0335.

123. Censi R, Schuurman W, Malda J, *et al.*, 2011, A printable photopolymerizable thermosensitive p(HPMAm-lactate)-PEG hydrogel for tissue engineering. *Advanced Functional Materials*, vol.21(10): 1833–1842.
http://dx.doi.org/10.1002/adfm.201002428.

124. Jackson N and Stam F, 2015, Optimization of electrical stimulation parameters for electro-responsive hydrogels for biomedical applications. *Journal of Applied Polymer Science*, vol.132(12): 41687(1–8).
http://dx.doi.org/10.1002/app.41687.

125. Giani G, Fedi S and Barbucci R, 2012, Hybrid magnetic hydrogel: A potential system for controlled drug delivery by means of alternating magnetic fields. *Polymer*s, vol.4(2): 1157–1169.
http://dx.doi.org/10.3390/polym4021157.

126. Pati F, Jang J, Ha D-H, *et al.*, 2014, Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. *Nature Communications*, vol.5: Article Number 3935.
http://dx.doi.org/10.1038/ncomms4935.