CHAPTER 1

Overview of Injectable Hydrogels for 3D Bioprinting and Tissue Regeneration

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1.1 General Introduction

One of the major clinical issues related to accidental injuries among the aging population is the development of soft and hard tissue defects or damages.[1,2] Clinically, the patients are treated with surgical reconstruction, organ transplantation, artificial implants, *etc.*[3] Even though allograft or autograft transplantation is the most commonly used method, donor shortage and donor morbidity are still very important issues.[4] Owing to such limitations, the number of patients waiting for transplants grows annually, and they require alternative treatment methods to avoid such a long wait.[5,6] Recently, 3D bioprinting has received noticeable attention, and it has progressed vastly with the advancing technologies and materials.[7,8] Generally, the diseased or damaged tissue or organ is identified and further scanned using magnetic resonance imaging (MRI) or micro-computerized tomography (μ-CT) scans. Then, the images are reconstructed using specific software to obtain the 3D models.

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Further, these 3D models are converted to G-codes using a slicing software. These G-codes are then uploaded into 3D printers and printed on demand using biocompatible materials or the patients' cells isolated from bone marrow, adipose tissue or other sources that can be used in the bioinks, which consist of hydrogels encapsulating both the stem cells and the bioactive molecules.[9,10] 3D bioprinting by additive manufacturing is one of the most modern, promising technologies for scientists to develop complex tissues and organ constructs.[11–13] With these modern biofabrication techniques, the researchers can create tissue structures similar to the native tissues or organs with biomimetic functionalities. The 3D bioprinting process generally involves the deposition or addition of live cell added hydrogel (bioinks) biomaterials in well-arranged layers to obtain a 3D construct, which is capable of creating functional tissues or organs.[11,12] The desired 3D design and structural pattern are generally developed using computer-aided design (CAD) models. The whole 3D bioprinting process requires novel polymeric biomaterials, design software, coding software, movement controllers in 3D or 4D axis, sterile environment, *etc.*, apart from the live cells that are the primary constituent of the bioink.[9] This method enables us to create more intricate structures with high precision and control as well as homogeneous cell distribution, which are normally not possible in the other scaffold preparation methods reported previously. This technique has many advantages such as high precision, well-ordered pore structures, vast and complicated designs, fast process and even patient-specific customization with less time.[13–15] This helps in personalized medicine as well, where the individual's organ or damaged tissue can be scanned using MRI or μ-CT scans, and subsequent images can be ordered and processed in specific software to obtain 3D models. Further, these 3D models can be processed on demand and used to print economically viable patient-specific 3D constructs with high precision and customization within a short time period.[16–18]

Even though there are several 3D printing techniques reported in the literature, only a handful of techniques is routinely used in 3D bioprinting or biofabrication for tissue engineering and regenerative medicine as well as drug screening. These are mainly pneumatic or screw-based extrusion printing techniques such as direct ink writing (DIW), laser-induced forward transfer, inkjet bioprinting, stereolithography (SLA) and finally selective laser sintering (SLS).[11,19] In DIW, the bioinks containing cells and hydrogels are extruded from a nozzle to create the desired 3D structures using the preloaded 3D models. However, in inkjet printing, at high shear rates, the low-viscosity bioinks are deposited or added as droplets. The other methods mentioned earlier require light-curable or laser-reactive groups in the biomaterial to make the 3D structures. Also, they need sophisticated instruments for the printing process. Apart from these methods, several techniques such as electro-hydrodynamic printing, microwave- and acoustic-based bioprinting have been reported recently for 3D bioprinting.[11,13,20]

For all the 3D bioprinting techniques, the primary component is the bioink containing cells in normal hydrogels to produce engineered/artificial live tissues. The bioinks are the heart of the 3D bioprinting; hence, the selection of the

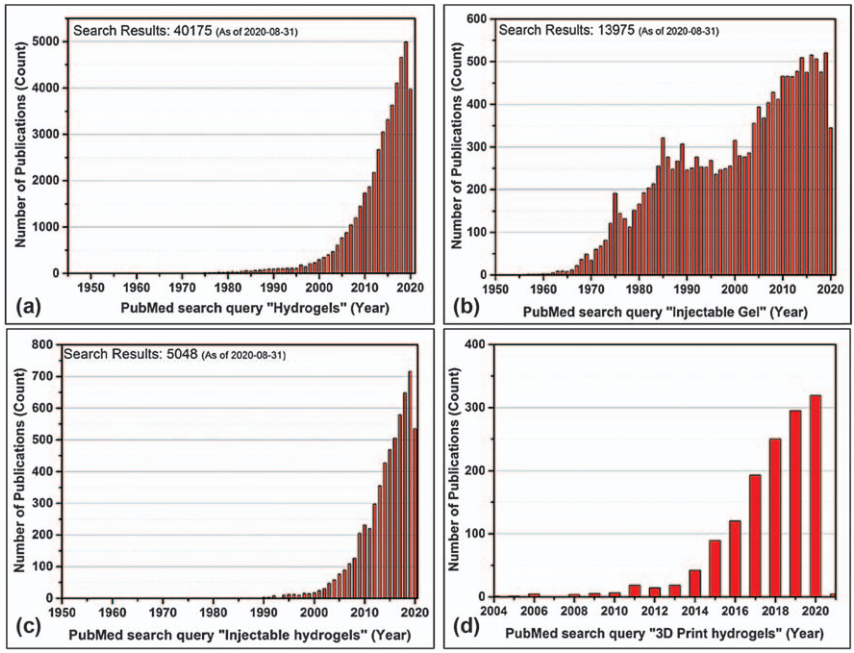

Figure 1.1 Number of search results (papers) obtained from PubMed search engine with different keywords: (a) Hydrogel, (b) injectable gel, (c) injectable hydrogels and (d) 3D print hydrogels.

bioink is highly critical to obtain the desired results. In bioink preparation, the hydrogels play a dominant role by enveloping cells for the protection of cells during bioprinting.[12,21,22] The hydrogels mimic the biological and physicochemical properties of the native tissues and provide the necessary environment for cells to live, grow and proliferate, leading to tissue regeneration.[23–26] They offer a convenient environment by providing proper nutrient transport, waste removal, oxygen–carbon dioxide exchange and cell attachment site. The hydrogels can be easily modified through chemical modifications, and their rheological, degradable or mechanical properties can be altered.[27] Apart from these reasons, these hydrogels exhibit shear thinning properties even while accommodating the live cells, without significantly affecting them.[28] Therefore, it becomes one of the ideal candidates for bioink preparations that can be used in 3D bioprinting.[29,30] This book provides more in-depth information about hydrogels, their various forms and synthesis methods, the characterization of different properties that can be applied in 3D bioprinting for tissue regeneration and clinical applications and the issues with intellectual properties and US-Food and Drug Administration (FDA) approval.

The emergence of 3D printing hydrogels and their importance can be evidently observed from the surge of research publications in recent years as shown in Figure 1.1d. ''Hydrogel'', ''injectable gel'', ''injectable hydrogels'' and ''3D print hydrogels'' were used as the search keywords in the PubMed

website, and the subsequent data were plotted from 1950 to 2020 to analyze the publication trend (Figure 1.1). The following sections give an overview of the contents of each chapter for an easy understanding of this book.

1.2 Introduction to Hydrogels and Their Syntheses

Hydrogels are three-dimensional (3D) networks of polymers, which are crosslinked and consist of hydrophilic polymer chains with the ability to absorb a huge amount of water.[31] The hydrogels possess the capacity to swell in water; however, these do not dissolve immediately.[32] The term ''hydrogel'' was first reported in 1894 even though it was a colloidal hydrogel consisting of inorganic salts; still, the term has been used consistently from that period.[33] The typical crosslinked hydrogel developed was first reported in 1960, and the polymeric material was poly(hydroxyethyl methacrylate) (pHEMA).[33] These hydrogels were developed mainly for application involving long-lasting contact with the human tissues and were considered to be the first man-made biomaterial used in the human body.[34] From this point, the research on hydrogels for biomedical-related applications started gaining momentum largely during the 1970s period. Over the years, the specific goals and aims changed constantly with the evolvement of the hydrogels and their different types through development in polymer chemistry and biomedical technologies. The history of hydrogels was explained as three different generations by Buwalda *et al.*[35] The first generation of hydrogels primarily consisted of gels with varying crosslinking methods by chemically modifying a monomer or polymer through an initiator. During this period, the idea was to create crosslinked materials that had high mechanical properties along with improved swelling properties through simple routes.[35]

Following this period, in the 70s, the importance of hydrogel shifted to the next level, where stimuli-responsive properties were inculcated in the hydrogels. These second-generation hydrogels were capable of responding to highly specific stimuli such as pH and temperature variations or biomolecule concentrations in a solution. The major advantage of such stimuli responsiveness is in enabling the hydrogels to be triggered for activating various processes such as releasing specific drugs, crosslinking/polymerization of the biomaterials and also to form *in situ* pores of gel networks.[33,35]

In the third-generation hydrogels, the focus shifted to the development of stereo complexed biomaterials and hydrogels linked using physical interactions. These evolutions led to the intensified attention of the scientific people to develop the modern ''smart hydrogels'', which has the capabilities to be tuned for obtaining specific properties including the stimuli responsiveness and controllable mechanical and other physico-chemical properties. This area of research is vast and limitless in its perspective application window, as it can be applied to any field from engineering to medical devices and many more.[33,35]

One of the pioneering works was reported by Wichterle and Lim on developing crosslinked hydrogels, which had the hydrophilic properties and

showed immense potential to be translated as a biocompatible material.[36] Such works led to much higher interest for the biomaterial scientists to foster these hydrogels for several years now. In continuation of this research, another notable contribution came from Lim and Sun, where they successfully encapsulated cells in calcium alginate microcapsule hydrogels.[37] In 1980s, synthetic burn dressings from hydrogels consisting of collagen and shark collagen as natural polymers were demonstrated by Yannas *et al.*[38]

Several hydrogels were reported by various scientists around the world after Yannas' report. Primarily, hydrogel polymers obtained from natural and synthetic sources were researched extensively for cell encapsulation because of their high biocompatibility, and later they are highly utilized in tissue engineering and regenerative medicine as scaffold matrices as they are mimicking the native extracellular matrices (ECM) and biodegrading over time.[24,39–42]

The hydrogels were crosslinked through either chemical or physical crosslinking. Among the chemical crosslinking methods, photopolymerization-based linking is one of the major methods used in injectable and 3D printing applications.[43] Chemical crosslinking agents are used to crosslink hydrogel's polymer networks commonly; however, because of the toxic effects of initiators and crosslinking agents on cells, they are generally not preferred in 3D bioprinting applications.[44] However, other methods including the Schiff's base reaction, hydrazone-based linking, Diels–Alder based crosslinking, azide–alkyne cycloaddition and enzyme-based crosslinking are also used in injectable and 3D printing applications.[29] Apart from these covalent linking methods, other non-covalent interactions such as ionic, hydrogen bonding, host–guest interactions, metal coordination, self-assembly peptide or peptide–DNA linkages are often reported for 3D bioprinting in the recent past.[9,45] However, one can select the appropriate method of gel crosslinking based on the requirements of the target tissues or organs. Selecting the appropriate method and suitable 3D printing technique is crucial to get the intended 3D construct with adequate mechanical and tissue-regenerative properties. This chapter highlights the different crosslinking mechanisms, their synthesis routes and finally their respective applications in 3D bioprinting.

1.3 The Characterizations of Hydrogels

The importance of the hydrogels and their application in 3D bioprinting and different tissue engineering areas show the significance of the hydrogels as biomaterials. Even though hydrogels are applicable in biomedical fields, they require appropriate properties according to the target tissues or organs.[7,8,46] Hence, the biomedical-related characterizations of the hydrogels for each application are highly essential. The novel properties of the hydrogels can be characterized by various instruments and methods.[47] The stability of the hydrogels, swelling properties, biodegradation, growth factor or drug loading efficiency, rheological properties, mechanical and physical properties including morphology, porosity, pore sizes, thermal degradation

properties and glass transition temperatures as well as different sol–gel transition through various methods are elaborated here in detail. In this chapter, the various properties of hydrogels are examined, and the characterizations of their properties are discussed in detail.

1.4 Natural and Nature-inspired Injectable Hydrogels

Biomimetic hydrogels have been highly researched by scientists in the recent decade, as they are developed from naturally existing polymers, which provide the required biological clues with their hierarchical and structural similarities. This dominance in research and its application is primarily due to the structural and chemistry level similarity with the native ECM in tissue engineering.[12,14,30,48] This offers the cells with proper attachment and proliferation environment. Furthermore, in case of *in vivo* injection with cells or without cells (only hydrogels), the natural existing polymers yield a variety of gelation mechanisms, which are not parallel to the synthetic biomaterials. Sol–gel transitions or sol–gel–sol transition can happen even with mild changes in the physical properties without affecting the incorporated cells. Even though several naturally derived biomaterials are researched and reported for injectable hydrogels or bioinks for 3D bioprinting, the importance of unmodified natural polymers still exists.[49,50] Different properties such as thermal responsiveness, polycompensation and ionotropic gelation are discussed for different natural polysaccharide biomaterials.

1.5 Self-assembling Hydrogels Based on Natural Building Blocks

Self-assembly processes of hydrogels exist in nature at nano, micro and macro levels.[51] It forms spontaneous assembly and well-ordered structures from numerous monomeric units without the help of any external forces. In self-assembly hydrogels, polymer-like fibrils are formed spontaneously from the self-assembly of the monomeric constituents through non-covalent molecular interactions. This further leads to fibril entanglements and finally results in gelation with the extended fibrillary networks in an aqueous solvent.[52,53] This has numerous merits compared to the covalently linked hydrogels, especially in both 3D bioprinting and tissue regeneration applications. The main advantage is that they are made from bio-inspired or biomimetic natural molecules such as amino acids, proteins, peptides, carbohydrates, oligonucleotides or other therapeutic agents.[54] Another important property of such self-assembly hydrogels is that they have high elastic recovery potential even after getting exposed to high shear rate of pressure. With adjustable rheological and other printing properties, they are deemed to be one of the promising biomaterials for tissue engineering.[55,56] The general introduction to the different self-assembled hydrogels and their possible uses in 3D bioprinting for tissue regeneration applications have been provided in this chapter.

For instance, several nano-fibrillar self-assembled peptide gels were reported, and their application in various biomedical fields was studied.[32,56] Depending on the specific functional group availability on the selected biomaterials, the self-assembled hydrogels can provide cell-specific interactions similar to the native tissues.[57,58] Silva *et al.* reported a scaffold material made from cell adhesive laminin derivative peptide sequences for increasing cell–tissue interactions.[59] They demonstrated this by using neural progenitor cells, which differentiated into its respective neuron lineage. Further, polypeptide-based hydrogels were investigated by Chilkoti *et al.*, which were similar to elastin peptide sequence. They were water-soluble and formed a gel-like aggregate when reaching a critical temperature.[60]

Similarly, peptide-based self-assembled hydrogels were prepared from commercially available synthetic precursors through ionic interactions.[61] The hydrogel provided the 3D environment needed for cell attachment and movement.[62,63] Also, the hydrogels showed exceptional printing ability for both soft and stiff constructs. Even live cell mixed gels were printed to show their potential for biofabrication.[64] Jian *et al.* 2019 reported an Fmoc-dipeptide based self-assembled hydrogel bioink for 3D bioprinting with adjustable mechanical properties and high degradability.[65] They used two contrasting dipeptides with different polarities and developed the bioinks for the bioprinting applications. These oppositely charged peptides formed gel *in situ* through electrostatic interactions during printing without the use of any crosslinkers. These gels showed high printability and cell viability when tested *in vitro.*

1.6 *In Situ* Forming Hydrogels

Hydrogels are considered as one of the potential biomaterials to deliver cells and therapeutics to the affected or diseased tissue area as they can protect the cells from the harsh environment. Mainly, the usage of *in situ* formed injectable hydrogels is gaining interest because of its minimal invasive administrable potential and the capability to reduce scar tissue development and the possible risk of infections.[66–68] As it is in injectable form, it can be advantageous in filling the damaged void areas of irregular tissue defects.

For this *in situ* formation of hydrogels, the particular hydrogel components should meet certain criteria like cell viability, cell retention, nutrient diffusion, waste removal and allowing soluble growth factors and biomolecules to transport.[69] Besides, they should not elicit any toxic effects to both the cells in bioinks and the surrounding tissues or cells; even the degradation products should not elicit or trigger an immune response. Further, controlled delivery of incorporated drugs or growth factors like bio-signals should be made possible through modifiable degradation rate. Above all, the injectable forms should facilitate a proper environment and protection to cells during injection, where high shear stresses will be experienced by the cells.[70,71] The shear thinning characteristics of the injectable hydrogels are crucial for this 3D bioprinting process, and the gels should have easily extrudable properties from a normal syringe with a suitable needle size. Once the gel releases from the needle, it

should form *in situ* gelation with the thermal stimuli or other means to encapsulate the cells.[72,73]

Similarly, *in situ* forming hydrogels are highly appropriate for 3D printing applications because of *in situ* gelation and their high biocompatibility properties. With these applications in mind, *in situ* gel forming synthetic and natural polymers are reported vastly. Even naturally derived materials in combination with synthetic materials are also reported.[74] Among them, synthetic *in situ* forming hydrogel polymers provide the required controllable properties (compressive moduli or stiffness, swelling properties, rheological and degradation) and high batch to batch homogeneousness. However, they have some limitations in case of cell attachment sites and proliferation ability. Contrastingly, natural materials and their derivatives yield high bioactivity properties such as cell attachment sites, proliferation and bio-signals, but they need extensive processing and chemical functionalization to make them more stable.[75] These *in situ* forming hydrogels are diverse in nature depending on the stimuli and other properties. Yet, several such systems are reported recently in tissue engineering applications with exceptional injectability, stability, cell compatibility and controllable degradation of *in situ* forming hydrogels. In this chapter, the multiple crosslinking methods for *in situ* forming hydrogel preparation and their present progress and future standpoints are reviewed.

1.7 Injectable Biopolymer Hydrogels for Regenerative Medicine

Previously, the properties of injectable hydrogels and *in situ* gel forming hydrogels and their crosslinking methods were highlighted. This chapter provides an overview of the different injectable biopolymer hydrogels and their specific applications in regenerative medicine. Apart from the synthesis methods and crosslinking mechanisms to develop injectable hydrogels, the biological functionalization of the different polymers is very crucial.[76] The functionalization of hydrogels for the particular target tissue is highly essential considering the diverse nature of the tissues and their *in vivo* functions.[76] Regeneration of tissues generally deals with the application of cells, especially stem cells, which can stimulate native tissue like regeneration without losing their original structure and functions.[77] However, in case of tissue repair, the damaged or diseased tissue will be filled with granulation-like tissue, which leads to the formation of scar tissue.[78,79] But, these repaired tissues will not have the same function like the actual tissue. Hence, tissue regeneration using viable stem cells is considered as the more promising approach in tissue engineering.[80,81] The recent updates and progress in the injectable and 3D bioprinting gel researches are elaborated in this chapter. Also, the application of such injectable hydrogels to functionally recover and regenerate the damaged or diseased tissues by incorporating various biological cues is also discussed and highlighted.

1.8 Hydrogels Processing Techniques and Vascular Tissue Engineering

Customary biofabrication techniques rely on the simple or composite polymeric building blocks. However, the scaffold should mimic the non-homogeneous native tissue with nanostructure level complexity. Generally, the scaffold should have controlled porosity, interconnectivity, pore size and nanofibrous diameter similar to the ECM structures.[82,83] Hence, to address and develop scaffolds with nanofibrous architecture, electrospinning is employed in tissue regeneration. The basics of electrospun hydrogel scaffolds and its application in tissue engineering are introduced in this chapter.

Electrospinning is one of the most versatile and convenient methods to obtain nanofibrous scaffolds with appropriate nanofiber diameter and porosity. It consists of three main units, namely high voltage direct current (DC) source, syringe pump with syringe holders and a collector ground plate.[84] A high DC voltage (+ charge) is given to the polymer–solvent solution present in the syringe through the metal needle. Due to this, the polymer solution gets charged, and the polymer jet forms a Taylor cone, and subsequently, the polymer jet undergoes instability and leads to an elongation process, where the polymer jet is dragged towards the collector and at the same time the solvent evaporates. This process leads to the development of nanofibers without the solvent, which randomly deposits onto the oppositely charged ground plate collector. Based on the distance, polymer flow rate, molecular weight (polymer), voltage, solvent, polymer concentration and collector distance, the nanofiber formation can be optimized. Also, by varying the deposition time, the thickness of the scaffolds can be controlled and optimized as per the tissue needs. The diameter of the nanofibers may vary from 10 to 1000 nanometers according to the conditions and set up.[85] Apart from the common electrospinning technique, other advanced versions with core–shell nanofibers or multicomponent materials or triple-layered nanofibers are also possible by using co-axial based electrospinning set ups.

This electrospinning approach is one of the frequently used techniques for scaffold preparation in tissue engineering as it mimics the ECM collagen fibrous architectures.[86] Even though synthetic polymers are highly reported, yet natural polymers and hydrogels biomaterials are also reported for tissue engineering applications and others. Hydrogels with diverse properties are used to form different nanofibrous scaffolds with desired nanofibers and porosity according to the target tissue.[87,88] Especially, vascular tissue engineering applications require different cell phenotypes to form the blood vessels with triple layers of cells.[89,90] Hence, the use of hydrogels to develop such vascular grafts is easier by combining the cells with nanofibrous scaffolds, and different layered structures can be developed. Small diameter vascular grafts with high precision can be produced using this method, which has been highly commercialized in many biomedical fields.[91] For example, Gupta and his group reported the development of vascular scaffold combining the hydrogels-like gelatin and oxidized carboxymethylcellulose.[92]

They optimized several electrospinning parameters and demonstrated the advantage of the electrospinning method to produce nanofibrous vascular grafts for tissue engineering. The scaffolds showed high cell viability and tuneable fiber diameter and pore sizes.[92]

1.9 Rheological Aspects of Hydrogel Processing

Characterization of the rheological properties of the injectable-based hydrogels is very crucial in both injectability and 3D bioprinting considering the specific application and its selection. Rheological assessment can be used to study three important parameters such as processability or injectability, mechanical architecture probing and to find the macroscopic performance of the biomaterials under different actual processing environments from liquid-like to solid-like evolution.[47,93,94] In case of injectability and 3D bioprinting, the shear thinning property of the hydrogels is very important, where the viscosity of the gel decreases under high stress rates. However, if the viscosity of the gel increases after experiencing high stress rates, then they are considered as shear thickening biomaterials and are not suitable for injectable gel or extrusion-based 3D printing methods. This can be found using the apparent viscosity *vs.* shear rate graphs obtained from the rheometer. Similarly, other properties such as storage moduli, loss moduli and elastic recovery can be determined from the rheological characterization of the hydrogels.[95–97] Also, one can find the injectability index of the hydrogels, not to mention the inclusion of power law index calculations using the data of flow rate, viscosity and stress rate, where the n values should be lower than 1 to confirm it as extrudable gel, where n, greater than 1, means shear thickening and if n is equal to 1, then they are considered like water.[98–100] In this chapter, the rheological characterization method and its relevance in analyzing the properties for injectable hydrogels or 3D bioprinting are highlighted, and an overview of different specific parameters corresponding to the viscoelastic properties is also discussed. Besides, the difficulties and issues faced during rheological characterizations are reviewed, along with the other new techniques such as non-contact method, local characterization of small samples and high throughput analysis.

1.10 Interface (Cell, Gel, Surface) and Biocompatibility in Gel Processing

Extrusion-based 3D bioprinting has been researched vastly due to its versatility and simple setup with a high potential for developing functional 3D tissue constructs. This 3D bioprinting method comprises the steps involving the addition or deposition of live cell-laden pre-crosslinked gel or polymeric hydrogel precursor solution for *in situ* gelation, followed by post-printing processing and subsequent stabilization of the printed structures.[11,13–15,21] Even though the bioink material is recognized as the primary requisite for developing the desired tissue construct irrespective of the 3D bioprinting

technique used, the interface between the hydrogels, cells and the extruder or the bioink dispenser during the printing process is crucial. Considering all three and their interactions may influence the final bioprinting quality or fidelity and the cell viability of the encapsulated cells in the bioinks. If the bioink used is too stiff during bioprinting, it may damage the cells due to high shear rate and result in poor cell viability.[101–103] If the bioink does not show enough interface compatibility with the metal needle, then the extrusion pressure needed in pneumatic or screw-based printer will be high. This also may damage the cells and result in reduced cell viability.[101] Hence, the interface compatibility between the bioink hydrogel, cells and the dispenser or needle is an important parameter to check. However, the interface varies with different polymers depending on their sources or gelation methods in 3D bioprinting. The interface issues of bioink printing such as single polymer based bioinks and their limitations along with the recent advances in the combination of polymers or additions of other additives or including multiple crosslinking strategies to impart more mechanical properties to address the limitations are also discussed and elaborated in this chapter.

1.11 Bioprinting Hydrogels and Tissue Engineering

Hydrogels have been utilized for 3D bioprinting of cell encapsulating bioink due to their ability to be processed at low temperatures and low shear forces that are favorable for cell encapsulation. The design of a hydrogel bioink requires the consideration of several key parameters such as the material formulation, the cell type(s) within the bioink and the 3D bioprinting methodology. For the selection of these parameters, it is very important to consider the biochemical and physical characteristics of the gel material as well as the potential effects of hydrogel's cell encapsulation and printing on cell behavior. Advances in hydrogel design and printing technology have ultimately enabled the fabrication of constructs for both 2D and 3D tissues as well as 3D hollow, solid and anatomical structures. The design parameters involved in the creation of hydrogel bioinks and the advances in the fabrication of biomimetic tissue engineering constructs using hydrogel bioprinting are overviewed and described in this chapter.

1.12 3D Bioprinting Hydrogel for Hard Tissue Regeneration

Several bioinks, their subsequent printing and post processing for the development of functional tissues have been thoroughly researched in the recent decade. Mainly, solvent-free bioinks made using aqueous-based combinations are highly biocompatible and are capable of integrating with the native tissue under clinical applications. Even though the use of hydrogel-based biomaterials is highly promising in 3D bioprinting applications, their mechanical properties or stiffness may limit their use for hard tissue application when applied directly. Hence, different novel hydrogels

with advanced crosslinking strategies and additives may be needed to attain the necessary mechanical properties to match the hard tissues like cartilage or bones. Also, the present limitations and possible methods used recently to improve the mechanical and other functional properties for hard tissue regeneration by 3D bioprinting hydrogels are discussed and elaborated in this chapter. Finally, the other prospects for refining the hard tissue regeneration through 3D bioprinting are highlighted.

1.13 3D-bioprinting for Engineering Complex Tissues and Vascularization

One of the major challenges of 3D bioprinting or any biofabrication technique is controlled vascularization inside the bioprinted complex 3D constructs. This is essential considering the nutrient supply to the regenerated tissue *in vivo*. However, intricate 3D structures prepared using 3D bioprinting methods are difficult to vascularize using the commonly available methods. Recently, a few researchers have tried to address the issues by implementing different novel strategies. 3D bioprinting and vascularization strategies are discussed and reviewed in this chapter.

1.14 Hydrogels–Blood Interactions

Blood contains numerous proteins, which are highly specific and reactive to different foreign bodies, leading to difficulties in predicting the consequences of a new biomaterial when implanted. The blood reacts fast due to the presence of various proteins and may lead to serious consequences in the body. Hence, proper care should be taken before implanting any foreign material inside the body. The blood components can readily react with the foreign material surface or its by-products, resulting in blood clots or thrombosis and then the failure of the implanted or injected biomaterial. This kind of protein adsorption process is one of the immunological responses to foreign substances. Also, when the blood is exposed to such foreign bodies comprising some antigens, then it may trigger serious inflammation or macrophage activation. Hence, to avoid such issues or reactions, careful examination of the injectable materials or testing the substance for hemocompatibility should be performed prior to the implantation or injection. Several methods such as thrombosis, partial thromboplastin time, coagulation, aggregation, platelet adhesion and hemolysis tests should be investigated systematically before injection or implantation of the foreign materials. Further, the present scenario of the hemocompatibility examinations based on different natural and synthetic hydrogel materials is discussed in this chapter. Especially, high emphasis has been given to the intrinsic anti-thrombogenic characteristics of different synthetic hydrogels with specific examples for finalization of the injectable materials before application inside the body. Further, the surface functionalization and modification methods and their examples are given briefly.

1.15 Immune Reactions to 3D Printable Hydrogels and Their Immunomodulation for Tissue Engineering

Any foreign body implanted or injected inside the body will receive a foreign body response; however, the response and its subsequent reactions depend on the biomaterial surface properties, chemistry, roughness, charge or other functionalities with antigen-like properties, *etc.* These cascades of reactions may affect not only the foreign body but also the surrounding tissues and result in damaging the healthy tissues. In extreme cases, they can also elicit high immunological response leading to blood clots and other serious consequences leading to death. Hence, before injecting or implanting the constructs inside the body, proper assessments should be carried out. If the inflammation and macrophage activations are tolerably less and reduce after the initial implantation time, then it may be applicable unless they elicit any drastic harmful effects to the patient. Many immunomodulation methods are employed to test the immunological responses for foreign bodies from the body to increase the local tissue acceptance and its subsequent tissue regeneration. However, the immunomodulation strategies differ according to the target tissue, host conditions, scaffold materials and so on.

Several injected hydrogels or implanted 3D bioprinting constructs may induce immune reactions and face consequences from the body through immunological responses. These reactions may negatively affect tissue growth or development. Hence, knowing the immunological properties of the injectable hydrogel or 3D printable bioink will help decide the application of particular materials *in vivo*. The relationship between the *in vivo* immune reactions and the subsequent tissue regeneration processes is elaborated in this chapter with specific examples. Further, different methods for the immunomodulation of gels for enhancing the tissue regeneration are discussed.

1.16 Application of Natural Hydrogels for Cell Therapy: Focus on Osteoarthritis

Regenerative medicine helps in replacing or repairing the tissues that are damaged or diseased. In regenerative medicine, cell-based therapies and tissue engineering offer more potential benefits for the aging populations.[104–106] Especially, the availability of different biomaterials with novel properties is highly applicable in osteoarthritis (OA) patients, which is a model case of injectable cell therapy hydrogel applications to tissue regeneration, and this approach is highly appreciated as one of the attractive and safe methods in hydrogel injection and arthropathy.[107] The articular cartilage, which occurs as a cover for the bones, gets degraded slowly and leads to this problem. This articular cartilage is responsible for the flexibility and motion of the bone joints by providing enough cushioning effect between the bone joints and prevents from joint locking or reduces the impact forces during walking or jumping. However,

this articular cartilage has very few regenerative properties; hence, once damaged, it will be very difficult to replace or regenerate it normally by the body.[108,109] In order to address this problem, regenerative medicine and tissue engineering approaches use natural and semi-synthetic hydrogel or glycosaminoglycan (GAG) based natural materials with cells or other bio-factors.[110] Even though several approaches are reported, still the prevention of cartilage degradation and further OA incidence is challenging. One of the promising, minimally invasive approaches to address this issue is the use of injectable hydrogels with live cells or other inflammation modulators and biomolecules. Injectable hydrogels formed using natural polymers are highly promising in relieving pain and inflammation reduction for many OA-affected patients.[110,111] The use of such injectable hydrogels with controllable properties is an effective treatment for articular cartilage degradation or damage. The overview of various tissue engineering and regenerative medicine methods and technologies is highlighted for OA treatment using injectable hydrogel systems.

1.17 Clinical Application and Regulation of Bioprinting Biomaterials Focusing on Hydrogels

3D bioprinting along with other recent advancements in various biomedical fields lead to a major advancement in many clinical applications in real time.[112] Hence, repairing or replacing damaged tissue or organ or drug testing are more promising examples in 3D bioprinting of bioinks in recent years. The high applicability is mainly due to its various advantages such as precise arrangements, high resolution and predefined structures, which can be built even with live cells similar to the native tissues.[7,11,13] Even though huge progress is made in this area, yet many approaches are still under research and development and did not transform to the clinical level. Therefore, the hydrogels used in this 3D bioprinting applications and their possible clinical translation as well as the current status of hydrogel research are discussed and elaborated in detail in this chapter.

1.18 Current Status of Commercialization of FDA-approved Hydrogels and Their Intellectual Properties

Even though several hydrogels are reported for tissue engineering and 3D bioprinting applications, not all apply to humans. The hydrogels or matrices should be properly tested using standard testing protocols, and it needs to undergo several stages before it gets approved. It also needs clearance from the FDA for human trials and further commercialization. Only a handful of hydrogel materials reaches the commercialization stage; however, still many hydrogels may be withdrawn after commercialization and due to negative effects. Therefore, proper preliminary testing and then rigorous *in vitro* (laboratory) and *in vivo* (animal model) experiments are necessary to assess

the effectiveness of the developed hydrogels or bioinks.[113–115] At the same time, novel methods and inventions of injectable hydrogels and 3D printable bioinks should be properly protected from intellectual theft for the advancement of biomedical industry. Hence, intellectual properties are very important for both scientific stimulus and commercialization.[116–118] The major areas of commercial applications of hydrogels include biomedical areas like tissue engineering, 3D bioprinting, drug delivery, injectable hydrogels, contact lenses, stents, cancer therapy, *etc.* Among the several reported hydrogels in recent years, many are in clinical stages, and a few hydrogels have been commercialized already for human applications in various areas.[119] Also, the market scenario, intellectual properties based on hydrogels and their applications are discussed in this chapter.

1.19 Concluding Remarks and Future Perspectives

The progress of injectable hydrogels has grown tremendously in recent years and has been used in many tissue engineering and regenerative medicine applications. In the last decade, the application of injectable hydrogels in 3D bioprinting has increased exponentially, due to its numerous advantages over other biofabrication methods. Several crosslinking strategies and methods are introduced for developing functionally advanced hydrogels, which are highly used in 3D bioprinting and tissue engineering as injectable gels. Apart from this, the hydrogels should satisfy several criteria like cell-interactions, immunogenic response, stability, controlled swelling, controlled *in vivo* degradability, biocompatibility and printability and fidelity in the case of 3D bioprinting. Likewise, several properties should be evaluated during the investigation. Even though various hydrogels are reported, still many challenges need to be addressed to make them highly acceptable for humans without toxic effects. Further, injectable and printable hydrogels have additional challenges in their application toward 3D bioprinting in technology, biocompatibility and tissue regeneration. As an example, researchers and physicians around the world work harder to make it realistic with the combination of advanced technologies and methods including 3D bioprinting, CRISPR, gene silencing, RNA interferences (RNAi), gene therapies, *etc.* However, the development of fully functional organs similar to the native ones is still under research and clinically not available. Nevertheless, many injectable hydrogels are promising as they are in clinical trials after the FDA's approval. In conclusion, it is noteworthy that the progress in the area of injectable hydrogels for 3D bioprinting is enormous, and one would expect the printed organs with functional properties soon. Also, the combination of research, development and commercialization of such technologies should go hand in hand to reach the clinicians and the patients. Affordability and availability are also one of the primary necessities for the same. Through proper IP protection and commercialization, this could be highly possible. We hope the scientists and physicians around the world would make it happen soon, and one can get the personalized body parts on demand in real time.

References

1. M. M. Schimke, S. Paul, K. Tillmann, G. Lepperdinger and R. G. Stigler, *Gerontology*, 2019, **65**, 174–185.
2. M. Qasim, D. S. Chae and N. Y. Lee, *Int. J. Nanomed.*, 2019, **14**, 4333–4351.
3. A. D. Anastasilakis, E. Tsourdi, P. Makras, S. A. Polyzos, C. Meier, E. V. McCloskey, J. Pepe and M. C. Zillikens, *Bone*, 2019, **127**, 401–418.
4. R. R. Betz, *Orthopedics*, 2002, **25**, S561–S570.
5. J. B. Schrock, M. J. Kraeutler, D. A. Houck, M. B. McQueen and E. C. McCarty, *Orthop. J. Sports Med.*, 2017, **5**, 2325967117704634.
6. G. Filardo, E. Kon, F. Perdisa, F. Balboni and M. Marcacci, *Int. Orthop.*, 2014, **38**, 1905–1912.
7. S. Vijayavenkataraman, W. C. Yan, W. F. Lu, C. H. Wang and J. Y. H. Fuh, *Adv. Drug Delivery Rev.*, 2018, **132**, 296–332.
8. S. V. Murphy and A. Atala, *Nat. Biotechnol.*, 2014, **32**, 773–785.
9. S. Ji and M. Guvendiren, *Front. Bioeng. Biotechnol.*, 2017, **5**, 23.
10. H. J. Jeong, H. Nam, J. Jang and S. J. Lee, *Bioengineering*, 2020, 7, 32.
11. J. Gopinathan and I. Noh, *Biomater. Res.*, 2018, **22**, 11.
12. K. Dzobo, K. Motaung and A. Adesida, *Int. J. Mol. Sci.*, 2019, **20**, 4628.
13. R. Levato, T. Jungst, R. G. Scheuring, T. Blunk, J. Groll and J. Malda, *Adv. Mater.*, 2020, 1906423.
14. R. D. Pedde, B. Mirani, A. Navaei, T. Styan, S. Wong, M. Mehrali, A. Thakur, N. K. Mohtaram, A. Bayati and A. Dolatshahi-Pirouz, *Adv. Mater.*, 2017, **29**, 1606061.
15. E. A. Guzzi and M. W. Tibbitt, *Adv. Mater.*, 2019, 1901994.
16. N. Yu, T. Nguyen, Y. D. Cho, N. M. Kavanagh, I. Ghassib and W. V. Giannobile, *Orthod. Craniofac. Res.*, 2019, **22**, 69–75.
17. Y. M. Elcin, *Stem Cell Rev. Rep.*, 2017, **13**, 319–320.
18. C. Arrigoni, M. Gilardi, S. Bersini, C. Candrian and M. Moretti, *Stem Cell Rev. Rep.*, 2017, **13**, 407–417.
19. F. Pati, J. Jang, J. W. Lee and D.-W. Cho, *Essentials of 3D Biofabrication and Translation*, Elsevier, 2015, pp. 123–152.
20. C. Mandrycky, Z. J. Wang, K. Kim and D. H. Kim, *Biotechnol. Adv.*, 2016, **34**, 422–434.
21. F. Pahlevanzadeh, H. Mokhtari, H. R. Bakhsheshi-Rad, R. Emadi, M. Kharaziha, A. Valiani, S. A. Poursamar, A. F. Ismail, S. RamaKrishna and F. Berto, *Materials*, 2020, 13, 3980.
22. A. Bhattacharyya, G. Janarthanan and I. Noh, *Addit. Manuf.*, 2020, 101639.
23. D. Das, H. Cho, N. Kim, T. T. H. Pham, I. G. Kim, E.-J. Chung and I. Noh, *Carbohydr. Polym.*, 2019, **207**, 628–639.
24. S. Bang, U.-W. Jung and I. Noh, *Tissue Eng. Regener. Med.*, 2018, **15**, 25–35.
25. D. Das, T. T. H. Pham and I. Noh, *Colloids Surf., B*, 2018, **170**, 64–75.
26. D. Das, H. T. T. Pham, S. Lee and I. Noh, *Mater. Sci. Eng., C*, 2019, **98**, 42–53.
27. N. Ashammakhi, S. Ahadian, C. Xu, H. Montazerian, H. Ko, R. Nasiri, N. Barros and A. Khademhosseini, *Mater. Today Bio*, 2019, **1**, 100008.

28. I. Noh, N. Kim, H. N. Tran, J. Lee and C. Lee, *Biomater. Res.*, 2019, **23**, 1–9.
29. J. Gopinathan and I. Noh, *Tissue Eng. Regener. Med.*, 2018, **15**, 531–546.
30. D. Chimene, R. Kaunas and A. K. Gaharwar, *Adv. Mater.*, 2020, **32**, 1902026.
31. T. Thambi, Y. Li and D. S. Lee, *J. Controlled Release*, 2017, **267**, 57–66.
32. D. Das and I. Noh, *Adv. Exp. Med. Biol.*, 2018, **1064**, 3–24.
33. S. C. Lee, I. K. Kwon and K. Park, *Adv. Drug Delivery Rev.*, 2013, **65**, 17–20.
34. D. G. Anderson, J. A. Burdick and R. Langer, *Science*, 2004, **305**, 1923–1924.
35. S. J. Buwalda, K. W. M. Boere, P. J. Dijkstra, J. Feijen, T. Vermonden and W. E. Hennink, *J. Controlled Release*, 2014, **190**, 254–273.
36. O. Wichterle and D. Lim, *Nature*, 1960, **185**, 117–118.
37. F. Lim and A. M. Sun, *Science*, 1980, **210**, 908–910.
38. I. V. Yannas, E. Lee, D. P. Orgill, E. M. Skrabut and G. F. Murphy, *Proc. Natl. Acad. Sci. U. S. A.*, 1989, **86**, 933–937.
39. A. S. Hoffman, *Adv. Drug Delivery Rev.*, 2012, **64**, 18–23.
40. S. Y. Lee, S. Bang, S. Kim, S. Y. Jo, B.-C. Kim, Y. Hwang and I. Noh, *Biomater. Res.*, 2015, **19**, 1–11.
41. Y. Du, H. Liu, Q. Yang, S. Wang, J. Wang, J. Ma, I. Noh, A. G. Mikos and S. Zhang, *Biomaterials*, 2017, **137**, 37–48.
42. S. Bang, D. Das, J. Yu and I. Noh, *Nanomaterials*, 2017, **7**, 328.
43. K. S. Lim, J. H. Galarraga, X. L. Cui, G. C. J. Lindberg, J. A. Burdick and T. B. F. Woodfield, *Chem. Rev.*, 2020, **120**, 10637–10669.
44. J. Y. Shin, Y. H. Yeo, J. E. Jeong, S. A. Park and W. H. Park, *Carbohydr. Polym.*, 2020, **238**, 1c00034.
45. A. GhavamiNejad, N. Ashammakhi, X. Y. Wu and A. Khademhosseini, *Small*, 2020, **16**, 2002931.
46. T. Jiang, J. G. Munguia-Lopez, S. Flores-Torres, J. Kort-Mascort and J. M. Kinsella, *Appl. Phys. Rev.*, 2019, **6**, 011310.
47. E. A. Kiyotake, A. W. Douglas, E. E. Thomas, S. L. Nimmo and M. S. Detamore, *Acta Biomater.*, 2019, **95**, 176–187.
48. F. Kabirian and M. Mozafari, *Methods*, 2020, **171**, 108–118.
49. G. Gillispie, P. Prim, J. Copus, J. Fisher, A. G. Mikos, J. J. Yoo, A. Atala and S. J. Lee, *Biofabrication*, 2020, **12**, 022003.
50. A. Shapira, N. Noor, M. Asulin and T. Dvir, *Appl. Phys. Rev.*, 2018, **5**, 041112.
51. N. N. Ferreira, L. M. B. Ferreira, V. M. O. Cardoso, F. I. Boni, A. L. R. Souza and M. P. D. Gremiao, *Eur. Polym. J.*, 2018, **99**, 117–133.
52. D. Kedracki, J. N. Abraham, E. Prado and C. Nardin, *Macromol. Self-assembly*, 2016, 193–229.
53. A. Rajbhandary and B. L. Nilsson, *Gels Handbook: Fundamentals, Properties and Applications Volume 1: Fundamentals of Hydrogels*, World Scientific, 2016, pp. 219–250.
54. L. Saunders and P. X. Ma, *Macromol. Biosci.*, 2019, **19**, 1800313.
55. B. O. Okesola, Y. Wu, B. Derkus, S. Gani, D. Wu, D. Knani, D. K. Smith, D. J. Adams and A. Mata, *Chem. Mater.*, 2019, **31**, 7883–7897.

56. C. A. Machado, I. R. Smith and D. A. Savin, *Macromolecules*, 2019, **52**, 1899–1911.
57. D. M. Ryan and B. L. Nilsson, *Polym. Chem.*, 2012, **3**, 18–33.
58. M. Zhou, A. M. Smith, A. K. Das, N. W. Hodson, R. F. Collins, R. V. Ulijn and J. E. Gough, *Biomaterials*, 2009, **30**, 2523–2530.
59. G. A. Silva, C. Czeisler, K. L. Niece, E. Beniash, D. A. Harrington, J. A. Kessler and S. I. Stupp, *Science*, 2004, **303**, 1352–1355.
60. A. Chilkoti, T. Christensen and J. A. MacKay, *Curr. Opin. Chem. Biol.*, 2006, **10**, 652–657.
61. C. Cofiño, S. Perez-Amodio, C. E. Semino, E. Engel and M. A. Mateos-Timoneda, *Macromol. Mater. Eng.*, 2019, **304**, 1900353.
62. T. L. Lopez-Silva, D. G. Leach, I.-C. Li, X. Wang and J. D. Hartgerink, *ACS Biomater. Sci. Eng.*, 2018, **5**, 977–985.
63. P. Chen, *Colloids Surf., A*, 2005, **261**, 3–24.
64. B. Raphael, T. Khalil, V. L. Workman, A. Smith, C. P. Brown, C. Streuli, A. Saiani and M. Domingos, *Mater. Lett.*, 2017, **190**, 103–106.
65. H. Jian, M. Wang, Q. Dong, J. Li, A. Wang, X. Li, P. Ren and S. Bai, *ACS Appl. Mater. Interfaces*, 2019, **11**, 46419–46426.
66. T. Thambi, V. G. Phan, S. H. Kim, T. M. D. Le and D. S. Lee, *New J. Chem.*, 2019, **43**, 18979–18982.
67. R. Dimatteo, N. J. Darling and T. Segura, *Adv. Drug Delivery Rev.*, 2018, **127**, 167–184.
68. J.-A. Yang, J. Yeom, B. W. Hwang, A. S. Hoffman and S. K. Hahn, *Prog. Polym. Sci.*, 2014, **39**, 1973–1986.
69. L. Cai, R. E. Dewi and S. C. Heilshorn, *Adv. Funct. Mater.*, 2015, **25**, 1344–1351.
70. Y. Tang, X. Tong, B. Conrad and F. Yang, *Theranostics*, 2020, **10**, 6035.
71. L. Teng, Y. Chen, Y.-G. Jia and L. Ren, *J. Mater. Chem. B*, 2019, **7**, 6705–6736.
72. T. Thambi, V. G. Phan and D. S. Lee, *Macromol. Rapid Commun.*, 2016, **37**, 1881–1896.
73. L. Klouda and A. G. Mikos, *Eur. J. Pharm. Biopharm.*, 2008, **68**, 34–45.
74. M. Teodorescu, M. Bercea and S. Morariu, *Polym. Rev.*, 2018, **58**, 247–287.
75. A. Hatefi and B. Amsden, *J. Controlled Release*, 2002, **80**, 9–28.
76. D. P. Huynh, M. K. Nguyen, B. S. Pi, M. S. Kim, S. Y. Chae, K. C. Lee, B. S. Kim, S. W. Kim and D. S. Lee, *Biomaterials*, 2008, **29**, 2527–2534.
77. M. Liu, X. Zeng, C. Ma, H. Yi, Z. Ali, X. Mou, S. Li, Y. Deng and N. He, *Bone Res.*, 2017, **5**, 1–20.
78. Y. Yeo, W. Geng, T. Ito, D. S. Kohane, J. A. Burdick and M. Radisic, *J. Biomed. Mater. Res., Part B*, 2007, **81**, 312–322.
79. Y. Gao, Z. Li, J. Huang, M. Zhao and J. Wu, *J. Mater. Chem. B*, 2020, **8**, 8768–8780.
80. B.-S. Kim and C.-S. Cho, *Tissue Eng. Regener. Med.*, 2018, **15**, 511–512.
81. M. Sepantafar, R. Maheronnaghsh, H. Mohammadi, S. Rajabi-Zeleti, N. Annabi, N. Aghdami and H. Baharvand, *Biotechnol. Adv.*, 2016, **34**, 362–379.

82. Y. Matsuzaki, K. John, T. Shoji and T. Shinoka, *Appl. Sci.*, 2019, **9**, 1274.
83. N. Abbasi, S. Hamlet, R. M. Love and N.-T. Nguyen, *J. Sci.: Adv. Mater. Devices*, 2020, **5**, 1–9.
84. R. A. Rezende, M. A. Sabino, J. de Andréa Dernowsek, F. de Albuquerque Vilalba, V. Mironov and J. V. L. Silva, *Int. J. Adv. Med. Biotechnol.*, 2018, **1**, 41–47.
85. A. Sensini and L. Cristofolini, *Materials*, 2018, **11**, 1963.
86. J. Fernández-Pérez, K. E. Kador, A. P. Lynch and M. Ahearne, *Mater. Sci. Eng., C*, 2020, **108**, 110415.
87. J. Gopinathan, B. Indumathi, S. Thomas and A. Bhattacharyya, *RSC Adv.*, 2016, **6**, 54286–54292.
88. M. M. Pillai, J. Gopinathan, B. Indumathi, Y. Manjoosha, K. S. Sahanand, B. D. Rai, R. Selvakumar and A. Bhattacharyya, *J. Membr. Biol.*, 2016, **249**, 813–822.
89. J. Han, P. Lazarovici, C. Pomerantz, X. Chen, Y. Wei and P. I. Lelkes, *Biomacromolecules*, 2011, **12**, 399–408.
90. C. Xu, R. Inai, M. Kotaki and S. Ramakrishna, *Tissue Eng.*, 2004, **10**, 1160–1168.
91. H. Ahn, Y. M. Ju, H. Takahashi, D. F. Williams, J. J. Yoo, S. J. Lee, T. Okano and A. Atala, *Acta Biomater.*, 2015, **16**, 14–22.
92. J. Joy, J. Pereira, R. Aid-Launais, G. Pavon-Djavid, A. R. Ray, D. Letourneur, A. Meddahi-Pellé and B. Gupta, *Int. J. Biol. Macromol.*, 2018, **107**, 1922–1935.
93. M. V. Ghica, M. Hîrjău, D. Lupuleasa and C.-E. Dinu-Pîrvu, *Molecules*, 2016, **21**, 786.
94. M. H. Kim, Y. W. Lee, W.-K. Jung, J. Oh and S. Y. Nam, *J. Mech. Behav. Biomed. Mater.*, 2019, **98**, 187–194.
95. M. Chopin-Doroteo, E. A. Mandujano-Tinoco and E. Krötzsch, *Biochim. Biophys. Acta, Gen. Subj.*, 2020, 129782.
96. N. Diamantides, L. Wang, T. Pruiksma, J. Siemiatkoski, C. Dugopolski, S. Shortkroff, S. Kennedy and L. J. Bonassar, *Biofabrication*, 2017, **9**, 034102.
97. N. Paxton, W. Smolan, T. Böck, F. Melchels, J. Groll and T. Jungst, *Biofabrication*, 2017, **9**, 044107.
98. M. Zhou, B. H. Lee and L. P. Tan, *Int. J. Bioprinting*, 2017, **3**, 003.
99. M. Zhou, B. H. Lee, Y. J. Tan and L. P. Tan, *Biofabrication*, 2019, **11**, 025011.
100. J. H. Chung, S. Naficy, Z. Yue, R. Kapsa, A. Quigley, S. E. Moulton and G. G. Wallace, *Biomater. Sci.*, 2013, **1**, 763–773.
101. R. Chang, J. Nam and W. Sun, *Tissue Eng., Part A*, 2008, **14**, 41–48.
102. C. Colosi, S. R. Shin, V. Manoharan, S. Massa, M. Costantini, A. Barbetta, M. R. Dokmeci, M. Dentini and A. Khademhosseini, *Adv. Mater.*, 2016, **28**, 677–684.
103. K. Hölzl, S. Lin, L. Tytgat, S. Van Vlierberghe, L. Gu and A. Ovsianikov, *Biofabrication*, 2016, **8**, 032002.
104. N. Rajabzadeh, E. Fathi and R. Farahzadi, *Stem Cell Investig.*, 2019, **6**, 19.

105. B. Arjmand, M. Sarvari, S. Alavi-Moghadam, M. Payab, P. Goodarzi, K. Gilany, N. Mehrdad and B. Larijani, *Front. Endocrinol.*, 2020, **11**, 430.
106. Z. Liu, M. Tang, J. Zhao, R. Chai and J. Kang, *Adv. Mater.*, 2018, **30**, 1705388.
107. R. M. Vakharia, M. W. Roche, J. C. Alcerro and C. J. Lavernia, *Orthop. Clin.*, 2019, **50**, 415–423.
108. L. Roseti, G. Desando, C. Cavallo, M. Petretta and B. Grigolo, *Cells*, 2019, **8**, 1305.
109. N. Nasiri, S. Hosseini, M. Alini, A. Khademhosseini and M. B. Eslaminejad, *Drug Discovery Today*, 2019, **24**, 2212–2224.
110. Z. He, B. Wang, C. Hu and J. Zhao, *Colloids Surf., B*, 2017, **154**, 33–39.
111. K. L. Spiller, S. A. Maher and A. M. Lowman, *Tissue Eng., Part B*, 2011, **17**, 281–299.
112. I. Matai, G. Kaur, A. Seyedsalehi, A. McClinton and C. T. Laurencin, *Biomaterials*, 2020, **226**, 119536.
113. S. Cascone and G. Lamberti, *Int. J. Pharm.*, 2020, **573**, 118803.
114. A. Mandal, J. R. Clegg, A. C. Anselmo and S. Mitragotri, *Bioeng. Transl. Med.*, 2020, **5**, e10158.
115. S. V. Murphy, A. Skardal and A. Atala, *J. Biomed. Mater. Res., Part A*, 2013, **101**, 272–284.
116. H. A. Quazi, *Commercializing Nanotechnology: A Roadmap to Taking Nanoproducts from Laboratory to Market*, CRC Press, 2020.
117. O. L. Lanier, K. G. Christopher, R. M. Macoon, Y. Yu, P. Sekar and A. Chauhan, *Expert Opin. Drug Delivery*, 2020, **17**, 1133–1149.
118. A. Elemoso, G. Shalunov, Y. M. Balakhovsky, A. Y. Ostrovskiy and Y. D. Khesuani, *Int. J. Bioprint.*, 2020, **6**, 301.
119. J. Gopinathan and I. Noh, *Biomimetic Medical Materials*, Springer, 2018, pp. 377–399.