AMERICAN SCIENTIFIC PUBLISHERS
Copyright © 2020 American Scientific Publishers
All rights reserved
Printed in the United States of America

Review
Journal of Biomedical Nanotechnology
Vol. 16, 1667–1686, 2020
www.aspbs.com/jbn

Role of Hydrogels in Bone Tissue Engineering: How Properties Shape Regeneration

Yanting Wu[1], Xu Zhang[1], Qian Zhao[2], Bowen Tan[1], Xingyu Chen[3], and Jinfeng Liao[1,*]

[1] *State Key Laboratory of Oral Diseases, National Clinical Research Centre for Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu, 610041, P. R. China*
[2] *Department of Pharmacy, Eastern Hospital, Sichuan Provincial Medical Sciences Academy & Sichuan Provincial People's Hospital, Chengdu, 610101, P. R. China*
[3] *College of International Education, Beijing University of Chemical Technology, Beijing, 100029, P. R. China*

Bone defect that resulted from trauma, tumors, and other reasons is believed as a common clinical problem, which exists mainly in post-traumatic healing. Additionally, autologous/allogeneic transplantation, bone tissue engineering attracts increasing attention due to the existing problem of the limited donor. The applications of biomaterials can be considered as a rising and promising strategy for bone regeneration. Especially, hydrogel is featured with hydrophilic characteristic, good biocompatibility, and porous structure, which shows unique properties for bone regeneration. The main properties of hydrogel such as surface property, adhesive property, mechanical property, porosity, and degradation property, generally present influences on the migration, proliferation, and differentiation of mesenchymal stem cells exclusively or in combination, which consequently affect the regeneration of bones. This review mainly focuses on the theme: "how properties of hydrogel shape bone regeneration." Moreover, the latest progress achieved in the above mentioned direction is further discussed. Despite the fascinating advances researchers have made, certain potential challenges continue to exist in the research field, which need to be addressed for accelerating the clinical translation of hydrogel in bone regeneration.

KEYWORDS: *Hydrogel, Property, Osteoinduction, Mesenchymal Stem Cells, Bone Regeneration.*

CONTENTS

*Author to whom correspondence should be addressed.
Email: liaojinfeng.762@163.com
Received: 20 November 2020
Accepted: 28 December 2020

INTRODUCTION

Trauma, infection, tumor, and congenital diseases can cause severe bone defects [1]. As a common illness that could be seen in our daily lives, bone defects have received considerable critical attention. The trauma caused by severe bone defect not only reduces the life quality of the patient, but also makes their financial burden even heavier. For instance, the cost of treating bone defects in the United States can reach as high as $5 billion per year [2]. Therefore, bone defect is a big problem for ordinary families. Nowadays, the implantation of bone grafts [3], including autogenic, allogenic, and xenogeneic implantations have been constantly applied to the clinical treatment for bone regeneration [4]. Although these implantation methods have been used in decades, bone grafts still exhibit several limitations. For example, due to the limited source of allograft bone, the large-scale application of secondary surgery is restrained. Moreover, the use of transplanted bone still exhibits a relevantly high risk of immune rejection and surgical infections [5].

Yanting Wu, B.A. Bachelor of West China College of Stomatology, Sichuan University, China. She joined in State Key Laboratory of Oral Diseases, National Clinical Research Centre for Oral Diseases in 2017. And she focused on hydrogel in bone regeneration. She has joined in many research projects of Dr. Liao, and has been co-author in several papers. In recent years, her research has mainly included the application of biomaterials in bone tissue engineering.

Xu Zhang, M.D. a master of West China School of Stomatology in Sichuan University. Master Zhang was born in 1997. He received his Bachelor's degree from Nanjing Medical University in 2019. Later in 2020, he is studying for a master's degree in West China School of Stomatology under the supervision of Dr. Liao. Also, he passed the National Accreditation Test for Resident Doctors and got doctor qualification certificate in 2020. Recently years, his research interests are concerned with the application of hydrogel in bone regeneration.

Qian Zhao, M.D. pharmacist of Eastern Hospital, Sichuan Provincial Medical Sciences Academy & Sichuan Provincial People's Hospital, China. In 2015, she received her master degree of pharmaceutics from Sichuan university, China. Then she worked on the department of pharmacy of Eastern Hospital, Sichuan Provincial Medical Sciences Academy & Sichuan Provincial People's Hospital. Her research interest is in biomaterials and drug delivery system.

Bowen Tan, B.A. a bachelor of West China College of Stomatology, Sichuan University, China. Mr. Tan was born in 1999 and is chasing his bachelor's degree at Sichuan University. He is a member of the State Key Laboratory of Oral Diseases, National Clinical Research Centre for Oral Diseases. Recent years, his research interests are concerned with the application of biomaterials-based therapy of malignant solid tumors and bone regeneration.

Xingyu Chen, B.A. Bachelor of both Beijing University of Chemical Technology and the State University of New York College of Environmental Science and Forestry. Miss Chen was a research assistant in the IAR program from 2019 to 2020 and managed the courses of biotechnology, genetics, and basic background of vaccine and COVID-19. During that period, Ms. Chen taught biotechnology and essay writing for students. Miss Chen worked on a technique for predicting Acarbose treatment based on stratification of gut by using 16SrRNA sequencing and she published a patent in 2020. In recent years, her research interests are bioinformatics and drug delivery system.

Jinfeng Liao, Ph.D. Associate Professor of West China Hospital of Stomatology, Sichuan University, China. She received her Ph.D. under the guidance of Professor Zhiyong Qian from State Key Laboratory of Biotherapy, Sichuan University in 2015. Then she worked at West China Hospital of Stomatology, Sichuan University till now. She carried out postdoctoral work at the Hong Kong Polytechnic University under the supervision of Professor Pei Li from 2017 to 2019. Her research interests are in the area of biomaterials, tissue engineering, and tumor therapy. She has published more than 30 papers in peer-reviewed journals. Dr. Liao is in charge of some projects, including the National Natural Science Foundation (31972925, 31600778), Sichuan Science and Technology Program (2020YJ0065).

Therefore, the efficient strategy of bone regeneration is in great demand.

The bone tissue is composed of collagen fibers and mineral calcium phosphate, which normally present a typical nanocomposite structure [6]. It includes two parts, namely the osteocytes and extracellular matrix (ECM). As a non-cellular, three-dimensional (3D) macromolecular network, the ECM contains hydrated and acidic molecules [7, 8]. Due to abundant available resources and controllable biocompatibility from different designing plans, bone tissue engineering emerges as a promising alternative for bone regeneration. Scaffolds, as essential parts of it, play a vital role in treatment. Despite providing brackets for cells and vessels, scaffolds with ideal quality influence the behavior of cells, provide support to the newly-formed tissue, and degrade upon the completion of the bone regeneration naturally [9]. Moreover, scaffolds interact with bioactive factors to initiate the beginning of cell growth and proliferation [10]. Currently, scaffold materials for bone tissue engineering are classified into natural polymers, synthetic polymers, and bioactive ceramics based on their chemical composition. Among them, bioactive ceramic materials are characterized by high strength and resistance to deformation. However, they are brittle and resistant to degradation [11]. Natural polymers have better biocompatibility than synthetic polymers but are less strong [12]. Hydrogel is a valuable, naturally derived polymer (there are also hydrogels modified based on synthetic polyvinyl alcohol), which consists of a network of hydrophilic polymers with similar properties to the ECM, promoting cell adhesion, proliferation, and differentiation [13]. Through modification, the strength and toughness of the hydrogel are enhanced. The hydrophilic nature of the hydrogel makes it susceptible to degradation *in vivo*. The main ingredients of hydrogels include polyethylene glycol (PEG), chitosan, collagen, fibrin, and gelatin [14–16]. Additionally, hydrogels with decent biodegradable performance exhibit a reduced rate of rejection potential to certain organs compared with other materials [17–19]. Therefore, hydrogels with cross-linking networks [20] can be considered as a potential and promising material in bone regeneration.

Generally, the promoting mechanisms for bone regeneration in terms of bone tissue engineering can be classified as three types: (i) osteogenesis, (ii) osteoinduction, and (iii) osteoconduction (Fig. 1) to three types [21]: (i) osteogenesis, (ii) osteoinduction, and (iii) osteoconduction (Fig. 1). Osteogenesis refers to the injection of the planting seeding cells with hydrogels to serve the purpose of providing the necessary materials *in situ* directly. Osteoinduction contains the process that primitive cells are stimulated and developed into the bone-forming cell lineage. In bone tissue engineering, it often goes as taking the hydrogels to lead the migration of mesenchymal stem cells (MSCs) to migrate to the traumatic place, where MSCs can be differentiated into osteoblasts [22, 23]. After, the next step with the osteocytes differentiated osteocytes from osteoblasts settled in the trauma area in order to maintain bone homeostasis [24]. Finally, osteoconduction occurs when the hydrogels provide support for the bones to reborn and help the vessel to regenerate [25].

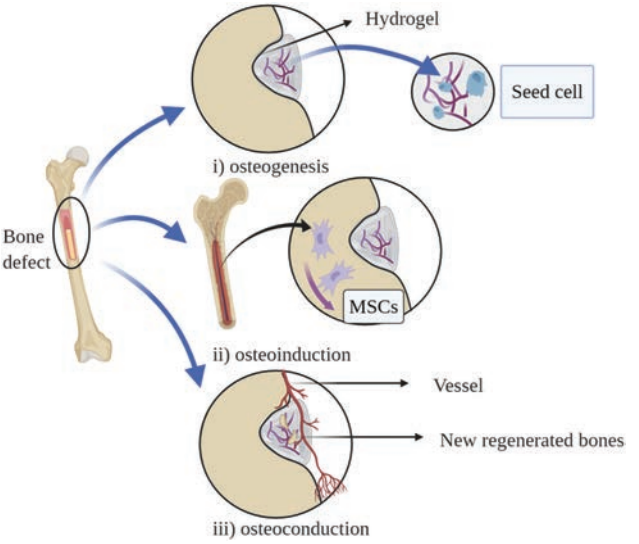

Figure 1. Three types of mechanism which can serve to promote the generation of bone: (i) Osteogenesis (planting seeding cells, materials and cytokines together packed in the scaffolds); (ii) osteoinduction (recruiting mesenchymal stem cells for bone regeneration); and (iii) osteoconduction (supporting and rebuilding new bones and vessels).

Inspired by the mechanism of bone regeneration in bone tissue engineering, the dependance on hydrogel's properties makes it possible to shape the bone regeneration through some adjustments (Fig. 2). Material sources used in hydrogel preparation are nontoxic and exhibits good biocompatibility [26, 27]. Moreover, hydrogels can attract and recruit MSCs to aid in bone regeneration. With the assistance of hydrogel and the growth factors that resulted from it, MSCs can be differentiated into osteoblasts. The diversity features of hydrogel surface are designated to assist the recruitment of MSCs and enhance the attachment of cells for bone regeneration. After planting the cells on the hydrogels surface, the cells will demand particular signals to adjust and regulate cell behavior [21, 28]. Hydrogels can also provide either biophysical or biochemical signals for MSC to conduct differentiation. Biophysical cues include features, such as the stiffness, modulus, and surface topography for hydrogels. Simultaneously, biochemical cues cover surface modification and bioactive factors, which will disperse in hydrogel [29]. Additionally, physical cues present significant importance during the interaction between the cell and the hydrogel. Such interaction is referred to as mechanotransduction, which reacts with the cell surface receptors and transfers into cells. After the information signals arrive at the cell nucleus, the behavior of cells will change accordingly. The immune reaction immediately gives responses after the *in vivo* implantation of hydrogel in the cell due to the rejection reaction. To reduce the immune responses, the applied hydrogels are required to be equipped with ideal biocompatibility, which consequently influence immune cells' behavior (behaviors such as the polarization of macrophages will happen). The selection of hydrogels for bone regeneration should also consider the balance degree between biodegradability and mechanical properties. For one reason, hydrogels with favorable mechanical properties support the defect areas of bone more effectively. Moreover, with outstanding biodegradability, the degradation of the equipped hydrogels will be more efficient and will offer bigger spaces for the newly regenerated bones [30]. However, as for the scaffold for the cells, the biodegradability of hydrogel often causes conflicts with the defected areas' stability. The reason for that phenomenon is that a good dissolved capability will result in poor performance on the hydrogels' mechanical properties [31]. Therefore, the balance between the above mentioned two properties should also be taken into detailed consideration.

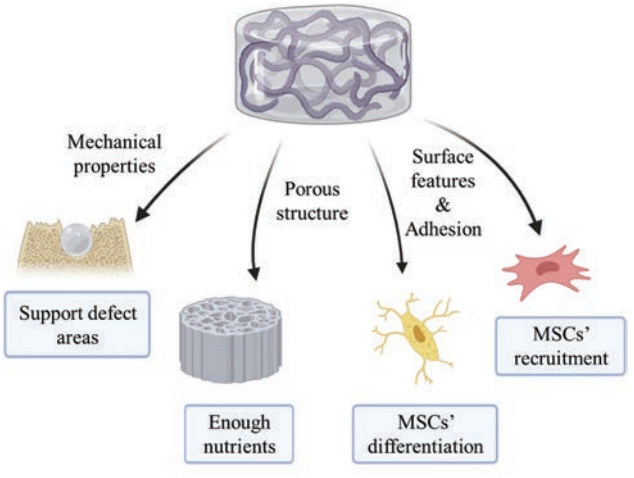

Figure 2. Features of hydrogel in bone regeneration and their functions.

In conclusion, the properties of hydrogels shape bone regeneration in an adjustable way. A preferable hydrogel that is suitable for the regeneration of bone can create an adaptable integrated microenvironment that combines with the body to harvest nutrients for the metabolism of cells [32]. The degraded hydrogel can provide space for cell seeding, and it can also interact with the internal environment as well as with specific mechanical signals. In this review, different hydrogels' properties were organized and summarized and their effects on bone regeneration were discussed and explained. Furthermore, recent research on the adjustment of these properties was also cited for providing different strategies to improve bone repair. Finally, future research directions regarding hydrogels' application for bone tissue engineering were proposed in this review.

THE MAIN PROPERTIES OF THE HYDROGEL

Hydrogels are characterized by surface property, adhesion, mechanical property, and porosity. The above-mentioned properties pose different effects in terms of the repair of bone defects. A diverse number of methods are applied for the purpose of enhancing or modulating these properties. The rest of this review is divided into four parts, which present a detailed description of the different effects that each method could have on the repair of bone. Moreover, this review will thoroughly introduce the preparation methods of relevant hydrogel material in recent researches.

Surface Properties

The surface properties of hydrogels usually cover three major features: wettability, roughness, and modification of surface with particular molecules. The three features are crucial to the proliferation and differentiation of MSC [33]. Through enhancing the similarity between hydrogels and ECM, the interfacial linkages can be significantly facilitated [34].

Hydrophilic/Hydrophobic Properties

The hydrophilic and hydrophobic properties of hydrogel are mainly dependent on their wettability. In order to measure the hydrophilicity/hydrophobicity of hydrogel, the contact angle analysis is commonly used to analyze the wettability of the hydrogel's interfaces. The contact angle

refers to the certain angle existing between the liquid-air plane and the solid–liquid interface, which is located tangent to the liquid-air plane, crossing the three-phase contact point [35]. Normally, the contract angle test will be performed with the contact angle analyzer under room temperature and within an appropriate humidity [36].

Recent researches provide evidence suggesting that hydrogel with hydrophobic feature may have a poor cellular attachment, while the surfaces of moderately hydrophilic features may have rich cellular attachment [37]. However, those cells tend to spread all over the hydrophobic surface of hydrogel. Certain hydrogels that are able to form some hydrophobic bonds with the cell surface may also help the cells to better colonize on hydrogel [38]. Thus, in regards of hydrophilic and hydrophobic effects on bone repair, perspectives may vary. Additionally, researchers also found that the biocompatibility would be improved along with the enhancement of hydrophilicity [39, 40]. Scientists have found at least three approaches to increase the hydrophilicity of hydrogels. First, the introduction of the charged groups into the hydrogel could improve the hydrophilicity of the hydrogel and decrease the contact angle [41]. Mizuro further found that the contact angle will be decreased when the absolute value of the charge which was carried by the hydrogel increased [42]. When the charge density reached 10 C/m^3, the contact angle will be reduced to approximately 40°, which indicated that the hydrophilicity of the hydrogel present has a significant increase. Usually, the charge could be increased by the addition of electrophilic and nucleophilic reagents to the hydrogel and upon adjustment of the pH value. For instance, Tan et al. added 2-(methacryloyloxy)ethyltrimethylammonium chloride (MAETAC) to polyethylene glycol di(ethylene glycol) (PEGDA), which is a component of polyethylene glycol [43]. In acrylate (PEGDA) hydrogels, hydrogels that possess both the features of hydrophilicity and the modulation of cell behavior can be obtained. However, Zhao et al. observed that the hydrogels which have been negatively charged performed to be detrimental to the adhesion of cell compared with that of the positively charged hydrogels [44]. Such performance may have a correlation relationship with the negatively charged surface proteins of the cell. Second, surface functionalization as a method to realize the target of modulating hydrophilicity of hydrogels has also received great attention from scientists in recent years [45]. The prepared hydrogels were usually immersed and stored in solution. Within a certain period of time, through the reaction of acylation and amination, the hydrophilic groups were grafted onto the surface of the hydrogel. Such an approach could significantly improve the hydrophilicity of the hydrogel. Third, the preparation of hydrogel with hydrophilic molecules also improves the hydrophilicity of the hydrogel. The molecules that are mainly studied in the third approach covered chitosan, HA, and PEG. Through the cross-linking of the above-mentioned molecules with other macromolecules, they could generate effects on other aspects of the properties. Chitosan is an excellent hydrophilic compound, which is mainly applied in drug delivery systems and bone tissue engineering. The hydrophilicity presented in this molecule resulted from numerous hydroxyl groups. Such properties could also be significantly enhanced when the crosslink action begins with other molecules. For example, Sabir's study confirmed that a ductile hydrogel with 90% CS and 10% PVA shows the need of a contact angle of less than 90°, proving that the hydrogels were equipped with good hydrophilic properties [46]. In other cases, increasing the hydrophilicity of hydrogels will also enhance the performance of other features. When Ikumi et al. designed one nHA/PLLA scaffold, its elastic modulus increased with the improvement of the hydrophilicity level [47]. They also found that in 2 weeks of the post-surgery, the bone repair in the nHA/PLLA group was remarkably greater compared with that of the control group. Similarly, Li et al. prepared 20 wt% nHA reduced graphene oxide (rGO) hydrogels [48]. Such hydrogel could promote the proliferation, adhesion, and osteogenic differentiation of MSCs. The photothermal effect of hydrogel also plays an important role in the treatment of tumors.

Roughness and Surface Topography

As the prominent properties of hydrogel surfaces, both roughness and topography pose great influence on the fate of cell fate and help regulate bone repair [49]. In general, the cause of roughness is often related with the microenvironment in which the biomaterial exists. It could pose effect on the adhesion level of cell, morphology, and signaling [50]. The surface topology often resemble with the physical cues that exist on the surface of the bone and gives a full play of its importance in osteoclast activity and osteogenic induction [51, 52]. Currently, atomic force microscopy (AFM) can be considered as the most widely used inspection technique in terms of measuring roughness and nanotopography [53]. To sum up, by means of a cantilever with a pointy tip, changes in surface properties (e.g., roughness) could be amplified into a laser signal. Measurements of the deviation among heights of peaks and valleys from the surface of the average curves can then reflect the roughness level of the hydrogel.

In 2011, Bigerelle et al. proved that hydrogels with the roughness of surface between 1.2 μm and 21 μm could influence the differentiation of MSC [54]. It has been observed that the above-mentioned roughness value range of the hydrogel could help increase the proliferation of cells and the formation of cytoskeletons [55] (Fig. 3(A)). Osteogenic differentiation could be further promoted through increasing the roughness of hydrogels [56, 57], which could also effectively enhance the expression of integrins, thus promote adhesion [58–60].

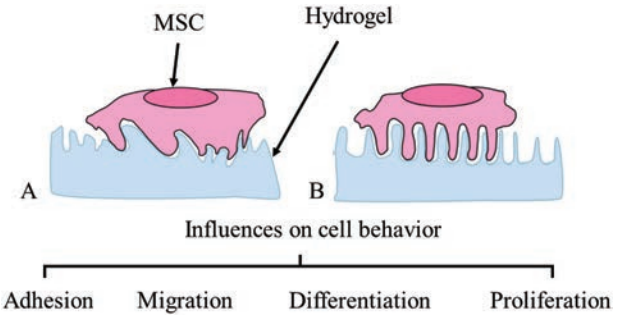

Figure 3. Cell responses in the biophysical microenvironment. (A) Roughness; (B) nanoparticle topology.

The reason for that phenomenon was partly rooted from the fact that a smooth surface would decrease cellular adhesion [58]. Therefore, MSCs prefer to adhere to rough hydrogels in order to maintain their stability. Additionally, the surface topography of the hydrogel can also help promote cell adhesion and further enhance the proliferation and differentiation of cell with the increase of the available surface area [61] (Fig. 3(B)). Meanwhile, matrix surface with great roughness could help promote the differentiation of osteogenic cell in MSCs [62]. Such property also led to questions regarding the disruption for cells on hydrogel interface [63]. In conclusion, increasing hydrogel roughness and depth of nanotopography could eventually facilitate osteogenic process [64].

Additionally, great efforts have been taken by the researchers in adjusting roughness and nanotopography of the surface to provide guidance to osteoinduction [65]. Li et al. developed silica nanoparticles (SNs), which are reinforced by the poly-citrate-siloxane hybrid elastomer (PCS-SN), and concluded that SNs could improve the survival rate of cells through the roughness of the surface [6, 21]. Another way to increase the irregularity and roughness of the hydrogel interface is to increase the sharpness of the edges [66, 67]. Moreover, researchers have found that in the nanoscale, sparse arrays could bring a better migration of cells with *in vivo* than that of dense arrays [68]. Currently, researchers have developed techniques, such as photolithography and 3D printing, in order to modulate the surface properties of hydrogels [69]. Photolithography functions with the introduction of the gelation of polymer solutions through patterned masks to form hydrogels with the desired shape. In recent years, preparation of hydrogels with increasing complex biodynamic characteristics is continually progressing with the study of 3D topography being made. Wai also proposed the optical maskless stereolithography (OMsL) technique, which can process 30-micron patterns on the surface of polyacrylic hydrogels [70] in a very short time. Also, in regards of the biological field, the most commonly used techniques are inkjet, extrusion, and laser-assisted printing. Prasopthum invented a 3D-printing technique on the basis of fast extrusion, which only takes one step to function for PCL/DAM. This method could also provide MSCs with good topographic cues to facilitate differentiation [71]. Recently, Jin et al. fabricated a hierarchical intrafibrillar mineralized collagen with a nanotopography similar to the natural bone surfaces and applied it to the synthesis of hydrogel. With transmission electron microscopy (TEM), scientists have found a distinctive D-periodic banding pattern on the surface of this hydrogel, which looks similar to the characteristics of bone tissue [72]. Altogether, the disorder on the interface makes a great contribution to osteogenesis.

Adhesion

Generally, adhesion can be classified into two types: the adhesion among cells and the adhesion between cells and ECM. They could function as mediators among cell–cell or cell-matrix adhesion [73], demonstrating as direct liaisons among the cell cytoskeleton and near cells, as well as proteins [74]. Although adhesion is closely related to the microscopic properties of the hydrogel, mechanical testing is chosen to be the testing method (Fig. 4). The following four methods are commonly used to conduct the test: probe-pull, lap-shear, 90° peel, and Bilayer-stretch, which represent and describe different aspects of hydrogel adhesion [75].

Connections between the cells can be mostly mediated by cadherin, which is a kind of a transmembrane receptor when the interactions are dominated by proteins, namely focal adhesions, nanoscale networks of integrins, and cytoplasmic proteins [51]. Adhesion gives full play to its vital role in the process of cell migrations and growth. If the interfaces between cells and hydrogels appear to be lacking of adhesion, the proliferation and growth of the cell will be affected and will present major phenomena, namely reduction, elongation, and migration [76, 77]. There are several ways that the adhesion property could affect the activities of cells. First, it could play as a bridge to connect cells with physical and biochemical cues to maintain the stability [78]. The hydrogel with good adhesive performance help attract cells and produce stable attachments. Second, there are several motifs that could help to form the adhesion property. One of the classic motifs is arginine-glycine-aspartic acid (RGD) motif [79]. Third, the adhesion of surfaces could interact with the wettability of hydrogel so that it could influence the growth of cells.

The adhesion of hydrogels shows certain relations with the chemical bonding, topography of the interface, and mechanics of dissipation. The chemical bonding of the contact interface is still often modulated by additional specific molecules. A commonly used peptide, RGD motif, which often located the interface matters, has certain influences on the adhesion between cells and hydrogels. Generally, such peptides operate to functionalize the interface so that its physiological environment could then be simulated. RGD can be considered as a signaling peptide mainly recognized by integrins and mediating the degree

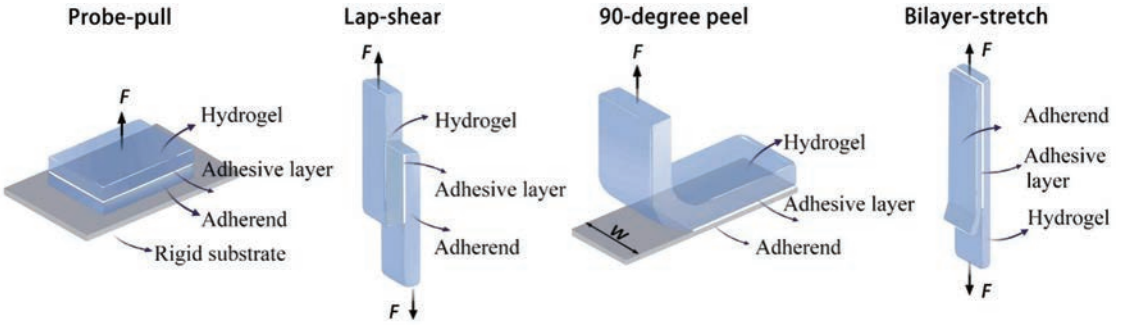

Figure 4. **Tests on characterizing adhesion.**

of adhesion in human body [80]. Integrin performs its function by the recognition and combination of several receptors [81]. Therefore, hydrogels with RGD peptides on the surface help promote their adhesive performance and provide MSCs with a more stable microenvironment to eventually promote the regeneration of bone. Liu et al. prepared PEG hydrogels, which are modified with RGD. They were convinced that the modification of RGD could significantly promote the biocompatibility of the hydrogels. Additionally, hydrogels with the decoration of RGD peptides would normally show better adhesion of cell compared with PEG hydrogels [82]. In 2018, Qu and his teammates combined RGD with hydroxybutyl chitosan (HBC) and designed an HBC-RGD hydrogel, which gained better benefits for adhesion and proliferation of BMSCs compared with the HBC hydrogel [83]. Meanwhile, Kim designed an MeGC hydrogel, which could increase the osteogenic differentiation of encapsulated BMSCs with RGD and amino acids *in vitro* [84]. Recently, Lee et al. proved that the application of UV light for the purpose of activating RGD peptides could cause the *in vivo* promotion of cell adhesion to biomaterials [85]. It means that with increasing number of adding RGD peptides, the adhesive ability is improved accordingly.

In spite of RGD, aptamer can also help enhance the adhesion level by several ways. An aptamer is a short, single-stranded nucleic acid molecule, which featured by affinity and specificity, wants to interact with the desired target [86]. The incorporation of aptamers with hydrogels takes free radical polymerization as an approach. Such incorporation could significantly improve the adhesion of hydrogels. Addition of the aptamer covers a wide range of specific adhesion of the cell type to the hydrogel. This review demonstrates the existence of a nominal increase in osteogenesis. Since properties of hydrogel, such as the mechanical property and porous structure of PEG-aptamers were not satisfactory, Zhang applied gelatin, nucleic acid aptamers, and polyethylene glycol to synthesize chimeric hydrogels as promising ECM mimics [87]. Additionally, Zhao also co-incorporated RGD and aptamers into the 3D-porous PEG hydrogels so that the promotion of cell adhesion, as well as blood vessel formation can be further completed, making an important contribution to the later stages of bone repair [88]. In addition to the specific molecule's incorporation, scientists have also exploited the physical effects of macromolecules in the process of promoting the adhesion of cells. Kaiwen Chen formed the chains of polyethylene glycol diethyl acrylate (PEGDA) networks with the non-covalent network, which contains highly diffuse giant PEGs to exploit the entanglement effect, which further promotes the adhesion level [89].

Additionally, the hydrophilic groups in accordance with the Schiff base reaction have exhibited the improvement of adhesion at the cell-hydrogel interface. The study on hydrogels has recently received sustained attention from scientists. Schiff reaction builds up the foundation of the cross-linking of amine and aldehyde groups to form a dynamic and covalent imine bond [90]. Schiff, which can be considered as base-bonds, could put every effort to form a network of hydrogels with cells and deposit of adhesion between hydrogels and cells. Inspired by the mussel, scientists have designed different adhesive hydrogels [91–95] with the ability and performance to adhere to various surfaces regardless of their smoothness and toughness. Based on the surface of mussel, polydopamine (PDA), hydroxyl groups, and amino groups have been applied to the training class of hydrogels in order to enhance the interface connectivity between cell-hydrogel [96, 97]. Recently, Zhou and his teammates designed a PPy-PDA hydrogel in order to promote adhesion. As more cells adhere to the surface, growth and proliferation of cells can be easily induced into the osteoblasts [98]. Han et al. developed a two-step PDA-clay-PAM hydrogel with adhesion. By oxidizing the hydroxyl group on PDA, the processed hydrogel provides a reproducible and adaptive adhesion level at the surface [92]. Additionally, changes in adhesion plaques could eventually stimulate and trigger β-catenin signaling to further help promote nuclear transformation and eventually enhance the expression of vinculin, especially in terms of rigid substrates [51].

Mechanical Properties

Mechanical properties are features of a material that provide mechanical strength and support cues required for cell

differentiation and proliferation [99]. The most commonly studied mechanical features of hydrogel are stiffness and elastic/compress modulus [100], whereby the universal test stand and indentation test are most often used to determine results [101]. The universal test stand converts load-displacement data into stress–strain data using simple geometric relationships [102], which are performed at a fixed rate until the specimen fails. The indentation test relies on a probe being pressed into the hydrogel before being immediately withdrawn. The remaining indentation is used as a measurement of the mechanical properties of the hydrogel [103]. By compressing the sample into a small area rather than the entire surface, it bypasses the usual requirement to process the hydrogel into the standard specimen shape. Furthermore, this technique eliminates issues with the mechanical gripping of the specimen as observed when using a regular specimen shape and prevents unnecessary waste of test material [101]. Generally, tissue regeneration is affected by mechanical signals. By sensing and interacting with hydrogel, mechanical signals and cells could have an influential relationship [21].

Regarding 2D- and 3D-scaffolds, a vast amount of research has determined the effects of mechanical signals on bone regeneration, especially on osteoinduction [104]. Discher et al. found that tissue cells have an active sense and response to the stiffness of the hydrogel [105]. It was also established that MSCs do not cover soft hydrogel efficiently, yet they spread evenly on stiff hydrogel (Fig. 5). Based on this, it was summarized that the extent of hydrogel stiffness results in varying differentiation for MSCs. Additionally, a study by Englar et al. identified that MSCs tend to differentiate into osteogenic lineages rather than adipogenic or myogenic ones when the mechanical properties were promoted. Hydrogels with an elastic modulus of 30∼35 kPa promoted osteogenic differentiation of MSCs, while those with elastic moduli less than 1 kPa promoted neurogenesis [106, 107]. This was believed to be due to the mimic performance between hydrogels and proposed tissue, which is closely linked to the cell phenotype [99, 108]. Overall, these findings suggested that hard hydrogels contributed to the bone regeneration process.

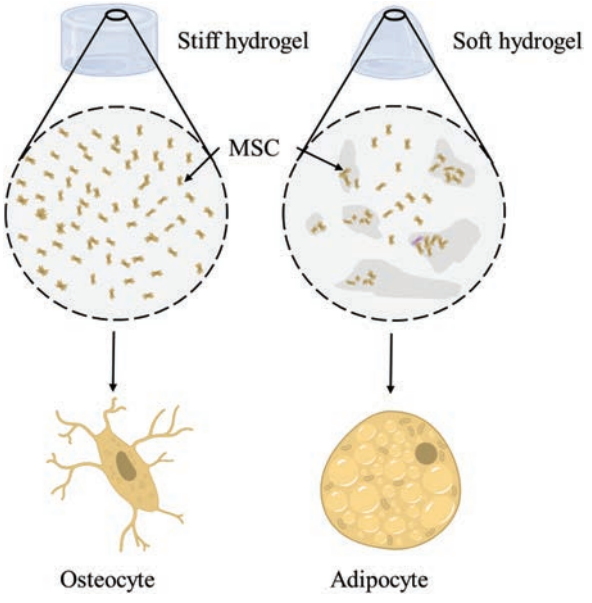

Figure 5. The spreading ability of MSCs on different forms of hydrogel. MSCs cover stiff hydrogel evenly but spread inconsistently on soft hydrogel.

The mechanical properties of hydrogels have an effect on the growth, proliferation, and differentiation of cells [30]. MSCs have been induced into osteoblasts when using stiff hydrogels [106]. This process uses signaling pathways, such as Rho/ROCK, FAK, and ERK1/2, mediated by α2-integrin [109]. Additionally, the nuclear transcription factor, YAP/TAZ, including a Hippo pathway, showed a direct connection with extracellular mechanical signals [110]. The optimum modulus provided adequate fixability to hydrogels to defend against environmental changes, particularly if joint trauma occurred [111] while also enabling sufficient toleration to maintain stability in bone regeneration. Alongside these findings, researchers have found that the elastic modulus affects the migration and proliferation of MSCs [112]. Mediated by myosin II A and II B, MSCs are capable of sensing differences in the matrix of the modulus. Stress fibers are cytoskeletal structures made of contractile actin bundles, responsible for cellular contractility and force. These structures were shown to undergo conformational changes while mediating MSC migration [113, 114]. This has been considered a directed migration as observed with anisotropic matrix [115, 116], in which the presence of an assembled microtubule network was necessary [117]. Vincent et al. discovered that MSCs preferred to accumulate on these types of high elastic modulus regions than the lower equivalents [116].

The doping of nanoparticles or microparticles results in further cross-linked bonds in the polymer network, which enhances the mechanical properties of hydrogel due to the complementation of the physical cross-linking of nanoparticles to the chemical cross-linking [30, 118]. This process mediates increases in the cross-linking/entanglement density of the polymer. Wu et al. designed double network hydrogels, where chemically cross-linked polyacrylamide (pAAm) is the main network doped with silica nanoparticles (SNPs) [119]. The use of nanoparticles led to a significant increase in the elastic modulus of hydrogels. Furthermore, Liao et al. developed an injectable calcium gluconate-alginate hydrogel with biodegradable porous poly(ε-caprolactone)-b-poly(ethyleneglycol)-b-poly(ε-caprolactone) microspheres (MPs/Alg) [120]. The inclusion of porous microspheres distinctly improves the mechanical strength of alginate hydrogel. Recently, polyacrylamide/lignin nanoparticle (PAM/LNP) nanocomposite hydrogels with derived lignin nanoparticles (LNPs)

have been studied and also presented impressive mechanical properties and improved biocompatibility [121]. Additionally, Zhai et al. doped nano-clay particles into PEG and through physical intercalation and chemical cross-linking within the PEG network, the resulting nanocomposite hydrogels showed enhanced mechanical properties compared to pure PEG hydrogels [122]. In another study, Xu et al. incorporated HAP nanoparticles into hydrogels, which was found to produce greater mechanical stability due to the resulting promoted cross-linking of the nanoparticles. HAP also provided unique chemical cues to promote osteoblast differentiation [123].

The mechanical properties can be regulated by changing the frequency of chemical reactions between the monomers. Within this field, double network hydrogels have been a primary focus of recent research, as cross-linkages can be increased by incorporating the molecules together [124]. Additionally, the balance between a hydrophilic and a hydrophobic state can affect the mechanical properties of hydrogels [125]. This balance relies on chemical cross-linking between specific groups of molecules. Liu et al. designed a hydrogel that generated physical entanglements through the interaction of gelatin methacrylate with multifunctional hydrogen bonds on tannic acid [126]. This newly created hydrogel had an ultimate stress value four times greater than that of GelMA alone. Another recent development has been the preparation of a hydrogel using biocompatible chitosan (CS) cross-linked with poly(*N*-(2-hydroxyethyl)acrylamide) (PHEAA), which uses covalent bonding of *N*-glucosamine units on CS. This hydrogel exhibited high tensile strength and strong elastic modulus (0.6 MPa) [127]. Furthermore, Wang et al. used both nanomaterial synergistic and chemical cross-linking to construct enhanced carbon/hydroxyapatite/PHEAA hydrogels, which showed stronger mechanical strength than hydrogels enhanced by nanomaterials or chemical cross-linking alone [128].

Porosity and Pore Shape

Properties, such as porosity, pore shape, and pore size, are highly dependent on the crosslink density, making them crucial in designing effective hydrogels. Indirect methods, such as gas adsorption testing and liquid immersion, and direct methods, such as microscopic imaging (scanning electron microscope (SEM) and TEM) and CT scanning, are used to measure porosity [129]. Gas adsorption is the most widely used method since it is a low-cost process and is used to measure the porosity of scaffolds with irregular pores [130]. Gas adsorption uses a gas molecule with a known molecular cross-sectional area to adsorb into a hydrogel under vacuum before the surface area and porosity are calculated based on the pressure equilibrium [131]. Nitrogen, argon, and carbon dioxide are the most commonly used gases for this method. Alternatively, SEM and TEM can be used to directly visualize the hydrogel pores, especially as SEM produces 3D images to accurately determine pore shape [132]. while TEM is prior to observing the nanopores. Liquid immersion and CT scanning methods are now less commonly used.

High porosity and large pores of hydrogels provide a larger surface area for MSCs to exchange substances [133, 134]. Lien and Matsiko found that a pore size of ~100 μm was optimum for cell attachment, migration, proliferation, and ECM production [135, 136]. Additionally, Zhao et al. demonstrated that hydrogels with pore sizes of 200–300 μm complemented the process of osteogenesis more effectively [137] compared to those with pore sizes of 100–500 μm. To build on these findings, Gao et al. created an injectable sodium alginate/PRP composite hydrogel with an average pore size of 200–300 μm [138]. Previous research suggested that the porosity above 80% was beneficial for cell proliferation and survival [139–143], while homogenous cell distribution was also identified at this level of porosity [144]. A great swelling ratio connects with great porosity and therefore, hydrogels with increased porosity will also display a superior degradative capability and permeability for water [145, 146]. In general, hydrogels with high porosity were more compatible for bone regeneration as the enhanced surface area of the hydrogels improves the capability of nutrient and waste exchange while also adsorbing osteogenic factor [147–150]. However, the observed advantages of high porosity and large pore size come at the expense of the mechanical properties and stability of the hydrogel [16, 147, 151]. Furthermore, Dey et al. demonstrated that the elastic modulus of hydrogel is dependent on the pore structures [152].

In order to reduce the adverse effect of high porosity on the mechanical properties of hydrogels, the method of increasing the crosslink density can be used [153]. Cross-linking density is related to the strength of the hydrogel and refers to the proportion of cross-linked structural units, which reflects the number of chemical bonds present. Additionally, cross-linking affects the network and pore size of hydrogels, which consequently influences cell phenotype and proliferation [154–156]. CS is often used to manufacture porous hydrogel [140, 141]. Grafting other groups onto CS could bestow superior properties to hydrogels and prevent other limitations. Liao et al. prepared a hydrogel composed of chondroitin sulfate methacrylate, methyl ether ε-caprolactone-acryloyl chloride, and graphene oxide, which presented appropriate porosity, pore size, and swelling capacity [157]. The enhanced porosity was attributed to the increased presence of CSMA. The ability of CSMA to exploit the heat-responsive gelling properties of gelatin means that the porosity can be controlled and cells can be homogeneously dispersed.

Previous studies have also found that various pore shapes influence cell behavior during bone repair [158, 159]. According to Phadake, cells in

rhombic pores show more advanced osteogenic differentiation [160]. Cylindrical and cubic pores were most conducive to differentiation into adipocytes, while cells in rectangular pores preferred chondrogenic differentiation [161, 162]. Moreover, in a three-dimensional context, random and sponge-like pores favored osteogenic differentiation as opposed to neatly laminated pores composed of cylinders. This may be due to the finer communication between the dispersed spongy pores compared to the lamellar pores. Ferlin et al. found that the cylindrical pores initially increased the expression of early osteogenic markers, although scaffolds with regular cubic pores increased protein expression levels in later stages [163].

Degradation Properties

Degradation is crucial to the structure and degradation of hydrogels [164]. By controlling the rate of degradation, researchers could regulate the release of drugs, while eliminating the hydrogel from the body when appropriate [165]. Usually, degradation is related to the molecular weight, cross-linker, crosslink density, stiffness, hydrophilicity, and the nature of the hydrolytic bonds of the hydrogel [164, 166]. Several stages are required for efficient degradation of hydrogels, including ester hydrolysis, enzymatic hydrolysis, photolytic cleavage, and reversible click reactions [167] (Fig. 6).

Several methods have been applied to characterize the degradation of hydrogels *in vivo* and *in vitro* settings. These methods are generally based on measurements of the properties of hydrogel samples taken at various time points. Initially, the concentration of the degradation products is measured. Bitter used the carbazole method to achieve this by measuring HA hydrogels degraded by hyaluronidase [168]. Second, changes in selected mechanical properties at specific time points are tested. Since the mechanical properties are correlated to the crosslink density, these properties can reflect adjustments in the crosslinked structure within the hydrogel [165]. Finally, most studies have used the weight loss of hydrogels to assess degradation.

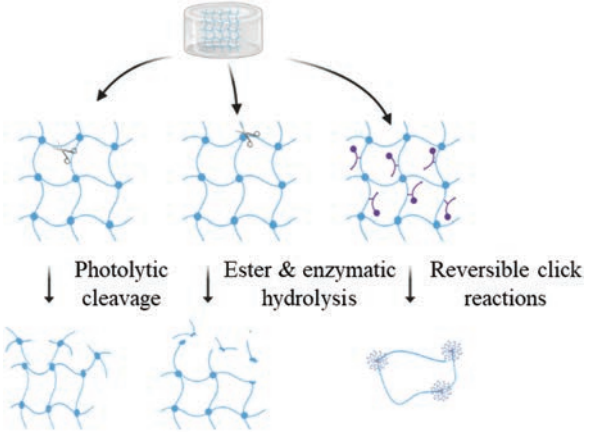

Figure 6. General mechanisms required for hydrogel degradation.

Bone regeneration can be promoted by degradation, reliant on the mechanical properties and cross-linking density of hydrogels [169]. When the rate of hydrogel degradation increases, the newly generated bone tissue displays more robust properties [170]. The balance between degradation and mechanical properties allows hydrogel to carry out roles appropriate to the stage of bone regeneration [167]. This is seen when hydrogel is initially needed to support the bone defect. Degradation of the hydrogel is later needed to allow adequate space for gradual increases in growth and at a later stage of the repair process, the hydrogel is required for vascular infiltration [171].

Generally, the degradation of materials is managed by several factors, such as by enhancing the hydrophilicity of the material, altering the raw materials and cross-linkers, using laser ablation, and controlling the enzymatic reaction. HA is a hydrophilic polymer that degrades rapidly in ECM and is commonly used to make biodegradable hydrogels. HA hydrogels have a porous structure with pore sizes ranging from 20 to 200 μm. They can also be degraded using hyaluronidase and reducing substances or when acidic. However, there are limitations to using HA hydrogels and the mechanical properties require improvement to become a more superior hydrogel material. Cui et al. prepared hydrogels modified with 3,3'-dithiodipropionate hydrazide and PEG diacetyl propionate for cross-linking, using a double network cross-linking method [172]. Hydrogel customized with maleimide-modified HA (MaHA) macromolecular monomers crosslinked with MMP-sensitive peptides was assembled. The result is a hydrogel that used adjustable crosslink density to regulate degradation and to guide the release of bone morphogenetic protein. Overall, this led to effective promotion of bone repair [173].

Further studies have focused on the enzyme-based degradation of hydrogels due to the uniqueness of the associated bio-derived properties. Kim et al. used lysozyme-doped CS hydrogel to modulate degradation, which induced adapted mechanical properties, pore size, and porosity of the hydrogel [174]. As a result of these adjustments, the hydrogel could undertake different roles in the early and late stages of osteogenesis. The modifiable degradation has also been shown to be effective for slow drug release. Microaggregates of alginate lyase has been encapsulated in alginate hydrogels to monitor the regulation of degradation, which has shown that it is possible to control the release of biological factors or cells using these enzymatic microaggregates [175]. This newly developed type of hydrogel is similarly useful for the infiltration of blood vessels in the late osteogenic phase.

Recently, the role of photodegradation in the generation of efficient hydrogels has attracted considerable attention.

Hydrogels have been used for laser-based degradation, whereby the hydrogels absorb laser to subsequently form plasma or by containing engineered photo-unstable groups. These processes are broadly used for cell and tissue engineering applications [176]. In a study by Brandenberg et al. lasers were irradiated onto poly(dimethylsiloxane) (PDMS) hydrogels to create complex microfluidic networks [177]. Notably, the researchers found that the product was compatible with 3D cell biology, but these networks could also be used to guide the migration and colonization of MSCs to facilitate bone repair. Furthermore, Hu et al. developed a thermally degradable hydrogel using the photothermal effect, which was capable of accurately controlling degradation of the hydrogel [178]. Thermal degradation can manage hydrogel stiffness and regulate the migration and differentiation of MSCs to advance bone regeneration.

HOW PROPERTIES SHAPE BONE REGENERATION

The Impact of Single-Nature Aspect Studies on Bone Regeneration

Individual properties of hydrogels, such as nanotopographies, stiffness, and the network structure, impact bone repair by influencing the microenvironment and cell surface signaling pathways.

Nanotopographies provide adhesive cues that influenced the adhesion of macrophages and the subsequent production of inflammatory cytokines [56, 57, 179]. It should be acknowledged that cells respond differently to physical cues. Most topological migration promotes the movement of cells to more dense substrate regions and is therefore detrimental to aggressive proliferation [107]. These factors were determined by two signaling pathways, PI3K and ROCK [158]. Additionally, nano topology can regulate macrophage differentiation toward M2, thus facilitating the formation of osteogenic cells [72]. Bone regeneration is a balance between bone formation and resorption. For bone repair to occur, bone formation must be the dominant process.

Hydrogel stiffness is one of the most important factors influencing the behavior of MSCs. Harder hydrogels provide cells with stable cellular focal adhesions, while softer hydrogels tend to result in dynamic cell adhesions [180]. Research by Balcioglu demonstrated that cell migration was linked to the matrix stiffness in a non-monotonic way. This suggested that moderately hard hydrogels usually induce preferable cell migration and contribute to osteoinduction [181]. This is mainly attributed to the MSC migration that occurs as a result of the binding of OPN to integrin $\beta 1$, following the activation of FAK and ERK pathways [182]. Recent studies have identified three series of actions that could cause this effect: (i) by adjusting the cytoskeletal tension, (ii) by controlling varied expression of signaling molecules, and (iii) by adjusting cell morphology based on the former two methods. Most microtubules present are a single tube and contribute to the formation of cellular structures [183]. The distribution of α/β tubulin in MSCs on hard hydrogels was broader than the distribution identified on soft hydrogels [51]. The greater area of distribution showed better cell proliferation and migration functions. To explain this process, Steinmetz et al. discovered that the dynamic mechanical stimulation adjusted MSC differentiation, which led to hyperexpression of collagen I. Consequently, this significantly encouraged migration, proliferation, and differentiation of MSCs [184]. To further develop these findings, Maisani et al. designed a composite hydrogel form of collagen I generated from a small synthetic amphiphilic molecule. As well as displaying distinctly improved mechanical and biological properties, this composite hydrogel enhanced the differentiation of MSCs into bone in the absence of osteogenic factors [185]. Other studies have developed a new class of ductile hydrogels based on the notion of collagen fibers and dual networks, which work well in osteochondral defects [186]. This form of tough hydrogel was formed using swim bladder collagen progenitors and poly(N, N'-dimethylacrylamide) (PDMAAm), which are responsible for the excellent mechanical properties it possesses. Moreover, it was found that the stiffness of hydrogel could trigger the expression of cytoplasmic β-catenin and enhance its nuclear translocation [51]. The Wnt/β-catenin signaling pathway has been established as being vital for bone maintenance through the regulation of osteoblasts [187, 188].

Lastly, the differences in network structure also influence bone regeneration. The network structures within hydrogels provide cell adhesion sites, while the pore structure provides adequate space for the proliferation and migration of cells [133]. It is possible that cell adhesion and migration could be modulated by regulating the 3D geometrical parameters [21]. The 3D network structure is partially reflected by the swelling behavior of the hydrogel, which in turn is influenced by the composition and cross-linking density [189–192]. Using this knowledge, a hydrogel was created using a pluronic ink with gelatin-methacryloyl (GelMA), to provide a 3D microenvironment for cells, which would ultimately enhance bone repair [193]. The resultant scaffolding showed good water absorption and was strongly resilient to damage. This is likely due to the stable traffic between the pores of this hydrogel, which would convey a level of resistance to changes in the external environment [120, 194–197]. Similarly, Gao et al. prepared PACG-GelMA hydrogels using acryloyl 2-glycine (PACG) and gelatin methacrylate (GelMA). The PACG-GelMA hydrogel presented strong mechanical properties and is swollen in promoting trabecular bone repair [198]. However, the mechanical properties and osteogenic irritation would decrease as the pore

size increased. As a consequence of this relationship, the hydrogel would prematurely undergo degradation before bone structures could be completely formed [149]. Moreover, Cheisy et al. discovered that nano-porous structures could influence macrophage responses. The bone immune environment promoted by 50 nm nanopores is conducive to the osteogenic differentiation of MSCs with optimal bone immunomodulatory effect. It has been suggested that this would lead to an increase in the osteogenic ability of bone biomaterial [71]. To test this, Kim et al. produced a hydrogel with a porous microstructure. By staining with ED-1 marker, they found that it effectively inhibited macrophage-mediated inflammation [199]. Kumar et al. also manufactured acrylic grafted tamarind polysaccharide-based hydrogels and found that the greater porosity of this type of hydrogel resulted in increased M2 cell expression, which is useful for tissue repair [24].

Additionally, it was shown that the pore structure has an essential role in cell migration [200]. In general, pores can influence macrophage morphology, migration, and most notably, the release of cytokines such as IL-6, which are accepted by osteoblasts and contribute to bone regeneration. Essentially, pores are involved in the transmission from physical cues to biochemical information [201]. Moreover, the *in vivo* degradation of hydrogels can influence pore size [202].

The Combined Effect of Two or More Properties on Bone Regeneration

Two or more hydrogel properties may act antagonistically or synergistically in regard to osteogenesis. Therefore, recognizing the nature of these interactions is a useful tool to regulate bone repair. Among these properties are porosity, the antagonistic relationship between swelling and mechanical properties, and the influence of surface properties and adhesion on the immune microenvironment.

The mechanical properties, swelling, and porosity represent the stability of the hydrogel and the level of support provided to organic tissue. Generally, the greater the crosslink density, the smaller the porosity and swelling. However, the mechanical properties improve [203]. A study by Stagnaro found that the porous structure of hydrogel collapsed in the range of smaller swellings [204]. Additionally, a rapid decrease in porosity was observed, which has an adverse effect on cell migration and bone repair. Nkhwa et al. found the addition of gelatin, calcium metaphosphate (CMP), and polyvinyl alcohol (PVA) allowed hydrogels to increase porosity without compromising the mechanical properties [205]. Cells were elaborately distributed over the entire surface of the hydrogel and formed a connected 3D network through the pores. Researchers found that the resultant mechanical properties of this modified hydrogel were suitable for low to medium load bone defects. Expanding on this, Wang et al. distinguished a hydrogel with high porosity and strong mechanical properties [206]. Poly(vinylphosphonic acid-co-acrylic acid) (PVPA- co-AA) hydrogel showed similar mechanical properties to bone by adjusting the content of PVPA, and was able to control the flow of nutrients due to the high porosity.

Further findings showed that surface properties and adhesion factors could affect the microenvironment *in situ*. The implantation of hydrogels induced inflammation, which transitioned from acute to chronic [207], and consequently reduced the stability of the hydrogels [8]. Of more recent interests are hydrogels with considerable immunomodulatory properties [208]. King et al. have demonstrated that once MSCs have settled in hydrogels, they can stimulate the production of angiogenesis and morphogenesis factors such as integrins to induce macrophage-promoted formation of M2 [209, 210]. From this, ossification occurs [211, 212]. Qiu et al. produced a hydrogel comprised of periosteal extracellular matrix (PEM) [213]. This creation induced macrophage recruitment and polarization to M2, which subsequently promoted osteogenic differentiation and vascular migration. Furthermore, this PEM hydrogel showed superior bone repair capacity in *in vivo* testing. Recent studies have shown that the adhesive cues of hydrogel are essential for macrophage functioning [211] and were found to promote osteogenesis by down-regulation of reactive oxygen species [214]. Additionally, high concentrations of parent molecules, including collagen and fibronectin, were shown to induce M2 polarization [215, 216]. Tanaka et al. found that fibrin hydrogels could progress the recruitment of macrophages and the secretory role of anti-inflammatory factors through M2, which resulted in enhanced osteogenesis [217]. Specifically, improved adhesion can promote MSC differentiation to accelerate osteoinduction. Thus, by adjusting the adhesive capability of hydrogels, the differentiation of MSCs can be controlled.

PROSPECTS AND CHALLENGES

The safety, efficiency, and controllability of biomaterials have allowed their use in clinical osteochondral repair systems, including Hyalonect®, Dexon™ by Medtronic, and Bioglass® [218]. Queiroz et al. compared the effects of hydrogel implantation with clinical cartilage grafting, in regard to the repair of cartilage defects [219]. The results found similarities between hydrogel and osteochondral bone grafting, but hydrogel implants were deemed to be safer, more effective, and less invasive to the patient. Further clinical developments in this field have been seen with the use of HA-based hydrogels loaded with BMP-2 for clinical skull repair [219], as well as the development of ECM-based hydrogel therapy. In the latter scenario, the properties of the hydrogel are adjusted to align those of the ECM, which is favorable for bone repair. However, the currently available hydrogel is composed of a single component, and there is a significant need to improve

the properties and application of this basic substance. The combined properties of hydrogels determine the effectiveness of bone repair. To produce hydrogels with optimum bone regeneration outcomes for further clinical use, the following recommendations are proposed to improve future hydrogel design and biomedical applications.

First, improving the osseointegration of the scaffold to the defect site needs to be considered. It is often assumed that a softer hydrogel will fit the bone defect better but at the expense of the support of the defect. Alternatively, harder hydrogels are not complementary to the bone defect. To resolve this issue, the multi-layer hydrogel technique can be used, where different levels of hydrogel stiffness are applied to different parts of the defect site. Second, injectable hydrogels are becoming a more prevalent area of research in recent years. The hydrogel is more fluid when injected and can precisely fit the damaged area of bone. Using these strategies results in improved mechanical properties after a period of time. Also, the relationship between adhesion and surface properties to osseointegration means that by improving adhesion and modulating surface properties, the contact between osteoblasts and the implanted hydrogel can be elevated. This would create an interlocking transition zone between the implant and the defect surface and would promote osseointegration.

A further consideration is the relationship between the degradable properties of the scaffold and the bone repair. Balancing the degradability and repairability of hydrogels should be further explored to achieve the desired bone repair. The biodegradability of hydrogels is linked to the porosity and pore features of the hydrogel, while the repair properties are related to the aforementioned components. Among them, mechanical properties and porosity are often opposing factors. Recently, the concept of multi-layered hydrogels has been proposed, whereby each layer of the hydrogel has porous components with different hardness and porosity characteristics. These components can be adapted to the specific site of bone injury, which makes them a highly promising development. Furthermore, 3D printing technology is advancing rapidly and hydrogels will eventually be designed and printed in 3D while maintaining different mechanical properties or porosities.

CONCLUSION

In summary, a systematic review of available research related to the adhesion, mechanical properties, interface, porosity, swelling, and pore characteristics of hydrogels has been undertaken. The properties of hydrogels can configure bone regeneration, and considerable progress has been made in finding the link between hydrogel properties and osteogenic induction. The properties of hydrogels are closely related to each other in various ways, depending on the biomaterial and the method used to manufacture each type of hydrogel. Significantly, these properties can interact to influence their overall effect on bone regeneration.

Acknowledgments: This work was financially supported by the National Natural Science Foundation (31972925), Sichuan Science and Technology Program (2020YJ0065), Sichuan University Spark Project (2018SCUH0029), State Key Laboratory of Oral Diseases (SKLOD202016), and National undergraduate innovation and entrepreneurship training program (C2020108366).

REFERENCES

1. Tian, T., Liao, J., Zhou, T., Lin, S., Zhang, T., Shi, S.R., Cai, X. and Lin, Y., **2017**. Fabrication of calcium phosphate microflowers and their extended application in bone regeneration. *ACS Applied Materials & Interfaces, 9*(36), pp.30437–30447.
2. Perez, J.R., Kouroupis, D., Li, D.J., Best, T.M., Kaplan, L. and Correa, D., **2018**. Tissue engineering and cell-based therapies for fractures and bone defects. *Frontiers in Bioengineering and Biotechnology, 6*, pp.1–23.
3. Luo, S., Wu, J., Jia, Z., Tang, P., Sheng, J., Xie, C., Liu, C., Gan, D., Hu, D., Zheng, W. and Lu, X., **2019**. An injectable, bifunctional hydrogel with photothermal effects for tumor therapy and bone regeneration. *Macromolecular Bioscience, 19*(9), pp.1–10.
4. Luby, A.O., Ranganathan, K., Lynn, J.V., Nelson, N.S., Donneys, A. and Buchman, S.R., **2019**. Stem cells for bone regeneration: Current state and future directions. *Journal of Craniofacial Surgery, 30*(3), pp.730–735.
5. Wang, Q., Xu, J., Jin, H., Zheng, W., Zhang, X., Huang, Y. and Qian, Z., **2017**. Artificial periosteum in bone defect repair—A review. *Chinese Chemical Letters, 28*(9), pp.1801–1807.
6. Li, Y., Guo, Y., Ge, J., Ma, P.X. and Lei, B., **2018**. In situ silica nanoparticles-reinforced biodegradable poly(citrate-siloxane) hybrid elastomers with multifunctional properties for simultaneous bioimaging and bone tissue regeneration. *Applied Materials Today, 10*, pp.153–163.
7. Theocharis, A.D., Skandalis, S.S., Gialeli, C. and Karamanos, N.K., **2015**. Extracellular matrix structure. *Advanced Drug Delivery Reviews, 97*, pp.4–27.
8. Sadtler, K., Singh, A., Wolf, M.T., Wang, X., Pardoll, D.M. and Elisseeff, J.H., **2016**. Design, clinical translation and immunological response of biomaterials in regenerative medicine. *Nature Reviews Materials, 1*, p.16040.
9. Pereira, H.F., Cengiz, I.F., Silva, F.S., Reis, R.L. and Oliveira, J.M., **2020**. Scaffolds and coatings for bone regeneration. *Journal of Materials Science-Materials in Medicine, 31*(3), p.27.
10. Jiang, T., Carbone, E.J., Lo, K.W.H. and Laurencin. C.T., **2015**. Electrospinning of polymer nanofibers for tissue regeneration. *Progress in Polymer Science, 46*, pp.1–24.
11. Gao, C., Deng, Y., Feng, P., Mao, Z., Li, P., Yang, B., Deng, J., Cao, Y., Shuai, C. and Peng, S., **2014**. Current progress in bioactive ceramic scaffolds for bone repair and regeneration. *International Journal of Molecular Sciences, 15*(3), pp.4714–4732.
12. Gasperini, L., Mano, J.F. and Reis, R.L., **2014**. Natural polymers for the microencapsulation of cells. *Journal of the Royal Society Interface, 11*(100), p.20140817.
13. Cai, Y., Zhang, J., He, Y., Li, Z., Hua, Y., Wu, Z., Gao, J., Ou, C. and Chen, M., **2018**. A supramolecular hydrogel of puerarin. *Journal of Biomedical Nanotechnology, 14*(2), pp.257–266.
14. Fan, D., Staufer, U. and Accardo, A., **2019**. Engineered 3D polymer and hydrogel microenvironments for cell culture applications. *Bioengineering (Basel), 6*(4), p.113.

15. Yeong, W.Y., Sudarmadji, N., Yu, H.Y., Chua, C.K., Leong, K.F., Venkatraman, S.S., Boey, Y.C. and Tan, L.P., **2010**. Porous polycaprolactone scaffold for cardiac tissue engineering fabricated by selective laser sintering. *Acta Biomaterialia, 6*(6), pp.2028–2034.

16. Vedadghavami, A., Minooei, F., Mohammadi, M.H., Khetani, S., Rezaei, K.A., Mashayekhan, S. and Sanati-Nezhad, A., **2017**. Manufacturing of hydrogel biomaterials with controlled mechanical properties for tissue engineering applications. *Acta Biomaterialia, 62*, pp.42–63.

17. Bilodeau, C., Goltsis, O., Rogers, I.M. and Post, M., **2020**. Limitations of recellularized biological scaffolds for human transplantation. *Journal of Tissue Engineering and Regenerative Medicine, 14*(3), pp.521–538.

18. He, Q., Zhang, J., Liao, Y., Alakpa, E.V., Bunpetch, V., Zhang, J. and Ouyang, H., **2020**. Current advances in microsphere based cell culture and tissue engineering. *Biotechnology Advances, 39*, p.107459.

19. Du, X.Y., Li, Q., Wu, G. and Chen, S., **2019**. Multifunctional micro/nanoscale fibers based on microfluidic spinning technology. *Advanced Materials, 31*(52), pp.1–38.

20. Peppas, N.A., Hilt, J.Z., Khademhosseini, A. and Langer, R., **2006**. Hydrogels in biology and medicine: From molecular principles to bionanotechnology. *Advanced Materials, 18*(11), pp.1345–1360.

21. Li, Y., Xiao, Y. and Liu, C., **2017**. The horizon of materiobiology: A perspective on material-guided cell behaviors and tissue engineering. *Chemical Reviews, 117*(5), pp.4376–4421.

22. Chen, Y., Shao, J.Z., Xiang, L.X., Dong, X.J. and Zhang, G.R., **2008**. Mesenchymal stem cells: A promising candidate in regenerative medicine. *International Journal of Biochemistry and Cell Biology, 40*(5), pp.815–820.

23. Fang, B., Liu, Y., Zheng, D., Shan, S., Wang, C., Gao, Y., Wang, J., Xie, Y., Zhang, Y. and Li, Q., **2019**. The effects of mechanical stretch on the biological characteristics of human adipose-derived stem cells. *Journal of Cellular and Molecular Medicine, 23*(6), pp.4244–4255.

24. Hitscherich, P., Nguyen, P.K., Kannan, A., Chirayath, A., Anur, S., Sarkar, B., Lee, E.J. and Kumar, V.A., **2018**. Injectable self-assembling peptide hydrogels for tissue writing and embryonic stem cell culture. *Journal of Biomedical Nanotechnology, 14*(4), pp.802–807.

25. Li, Q., Liu, C., Wen, J., Wu, Y., Shan, Y. and Liao, J., **2017**. The design, mechanism and biomedical application of self-healing hydrogels. *Chinese Chemical Letters, 28*(9), pp.1857–1874.

26. Place, E.S., Evans, N.D. and Stevens, M.M., **2009**. Complexity in biomaterials for tissue engineering. *Nature materials, 8*(6), pp.457–470.

27. Yahia, S., Khalil, I.A. and El-Sherbiny, I.M., **2019**. Sandwich-like nanofibrous scaffolds for bone tissue regeneration. *ACS Applied Materials & Interfaces, 11*(32), pp.28610–28620.

28. Argentati, C., Morena, F., Tortorella, I., Bazzucchi, M., Porcellati, S., Emiliani, C. and Martino, S., **2019**. Insight into mechanobiology: How stem cells feel mechanical forces and orchestrate biological functions. *International Journal of Molecular Sciences, 20*(21), p.5337.

29. Chen, Y., Sui, J., Wang, Q., Yin, Y., Liu, J., Wang, Q., Han, X., Sun, Y., Fan, Y. and Zhang, X., **2018**. Injectable self-crosslinking HA-SH/Col I blend hydrogels for in vitro construction of engineered cartilage. *Carbohydrate Polymers, 190*, pp.57–66.

30. Hsieh, W.T., Liu, Y.S., Lee, Y.H., Rimando, M.G., Lin, K.H. and Lee, O.K., **2016**. Matrix dimensionality and stiffness cooperatively regulate osteogenesis of mesenchymal stromal cells. *Acta Biomaterialia, 32*, pp.210–222.

31. Gu, Z., Huang, K., Luo, Y., Zhang, L., Kuang, T., Chen, Z. and Liao, G., **2018**. Double network hydrogel for tissue engineering. *Wiley Interdiscip Rev Nanomed Nanobiotechnol, 10*(6), pp.1–15.

32. Bhumiratana, S., Bernhard, J.C., Alfi, D.M., Yeager, K., Eton, R.E., Bova, J., Shah, F., Gimble, J.M., Lopez, M.J., Eisig, S.B. and Vunjak-Novakovic, G., **2016**. Tissue-engineered autologous grafts for facial bone reconstruction. *Science Translational Medicine, 8*(343), p.343ra83.

33. Wu, S., Dong, H., Li, Q., Wang, G. and Cao, X., **2017**. High strength, biocompatible hydrogels with designable shapes and special hollow-formed character using chitosan and gelatin. *Carbohydrate Polymers, 168*, pp.147–152.

34. Dinulovic, I., Furrer, R. and Handschin, C., **2017**. Plasticity of the muscle stem cell microenvironment. *Advances in Experimental Medicine and Biology, 1041*, pp.141–169.

35. Singh, A., Corvelli, M., Unterman, S.A., Wepasnick, K.A., McDonnell, P. and Elisseeff, J.H., **2014**. Enhanced lubrication on tissue and biomaterial surfaces through peptide-mediated binding of hyaluronic acid. *Nature Materials, 13*(10), pp.988–995.

36. Tavana, H., Simon, F., Grundke, K., Kwok, D.Y., Hair, M.L. and Neumann. A.W., **2005**. Interpretation of contact angle measurements on two different fluoropolymers for the determination of solid surface tension. *Journal of Colloid and Interface Science, 291*(2), pp.497–506.

37. Read, M.L., Morgan, P.B. and Maldonado-Codina, C., **2009**. Measurement errors related to contact angle analysis of hydrogel and silicone hydrogel contact lenses. *Journal of Biomedical Materials Research Part B-Applied Biomaterials, 91*(2), pp.662–668.

38. González-Carrasco, J.L., Cifuentes Cuellar, S.C. and Lieblich Rodríguez, M., **2019**. 5-metals. *Bone Repair Biomaterials (Second Edition)*, Second edn., edited by, K. M. Pawelec and J. A. Planell, Cambridge, Woodhead Publishing, pp.103–140.

39. Liu, S., Qi, D., Chen, Y., Teng, L., Jia, Y. and Ren, L., **2019**. Quadruple hydrogen bonds and thermo-triggered hydrophobic interactions generate dynamic hydrogels to modulate transplanted cell retention. *Biomaterials Science, 7*(4), pp.1286–1298.

40. Ikawa, H., Moroi, A., Yoshizawa, K., Saida, Y., Hotta, A., Tsutsui, T., Fukaya, K., Hiraide, R., Takayama, A., Tsunoda, T., Saito, Y. and Ueki, K., **2017**. Bone regeneration enhancement by ultra-violet (UV) treatment for uHA/PLLA absorbable mesh. *Journal of Cranio-Maxillo-Facial Surgery, 45*(5), pp.634–641.

41. Moroi, A., Okuno, M., Kobayashi, G., Gamo, H., Serizawa, I., Yoshizawa, K., Ikawa, H. and Ueki, K., **2018**. Effect on surface character and mechanical property of unsintered hydroxyapatite/poly-l-lactic acid (uHA/PLLA) material by UV treatment. *Journal of Biomedical Materials Research Part B-Applied Biomaterials, 106*(1), pp.191–200.

42. Schulz, A., Katsen-Globa, A., Huber, E.J., Mueller, S.C., Kreiner, A., Pütz, N., Gepp, M.M., Fischer, B., Stracke, F., von Briesen, H., Neubauer, J.C. and Zimmermann, H., **2018**. Poly(amidoamine)-alginate hydrogels: Directing the behavior of mesenchymal stem cells with charged hydrogel surfaces. *Journal of Materials Science—Materials in Medicine, 29*(7), p.105.

43. Mizuno, H.L., Tan, E., Anraku, Y., Sakai, T., Sakuma, I. and Akagi, Y., **2020**. Relationship between bulk physicochemical properties and surface wettability of hydrogels with homogeneous network structure. *Langmuir, 36*(20), pp.5554–5562.

44. Tan, F., Xu, X., Deng, T., Yin, M., Zhang, X. and Wang, J., **2012**. Fabrication of positively charged poly (ethylene glycol)-diacrylate hydrogel as a bone tissue engineering scaffold. *Biomedical Materials, 7*(5), p.055009.

45. Zhao, Y., Nakajima, T., Yang, J.J., Kurokawa, T., Liu, J., Lu, J., Mizumoto, S., Sugahara, K., Kitamura, N., Yasuda, K., Daniels, A.U. and Gong, J.P., **2014**. Proteoglycans and glycosaminoglycans improve toughness of biocompatible double network hydrogels. *Advanced Materials, 26*(3), pp.436–442.

46. Lee, S.Y., Lee, Y., Le, T.P., Oh, D.H. and Park, K.D., **2018**. Sulfobetaine methacrylate hydrogel-coated anti-fouling surfaces for implantable biomedical devices. *Biomaterials Research, 22*, pp.3–9.

47. Islam, A., Yasin, T., Gull, N., Khan, S.M., Sabir, A., Munawwar, M.A., Shafiq, M., Jamil, T. and Raza, M.H., **2016**. Fabrication

and performance characteristics of tough hydrogel scaffolds based on biocompatible polymers. *International Journal of Biological Macromolecules, 92*, pp.1–10.

48. Ikumi, R., Miyahara, T., Akino, N., Tachikawa, N. and Kasugai, S., **2018**. Guided bone regeneration using a hydrophilic membrane made of unsintered hydroxyapatite and poly(L-lactic acid) in a rat bone-defect model. *Dental Materials Journal, 37*(6), pp.912–918.

49. Li, D., Nie, W., Chen, L., McCoul, D., Liu, D., Zhang, X., Ji, Y., Yu, B. and He, C., **2018**. Self-assembled hydroxyapatite-graphene scaffold for photothermal cancer therapy and bone regeneration. *Journal of Biomedical Nanotechnology, 14*(12), pp.2003–2017.

50. Elias, C.N., Oshida, Y., Lima, J.H. and Muller, C.A., **2008**. Relationship between surface properties (roughness, wettability and morphology) of titanium and dental implant removal torque. *Journal of the Mechanical Behavior of Biomedical Materials, 1*(3), pp.234–242.

51. Wang, Q., Huang, Y. and Qian, Z., **2018**. Nanostructured surface modification to bone implants for bone regeneration. *Journal of Biomedical Nanotechnology, 14*(4), pp.628–648.

52. Xie, J., Zhang, D., Zhou, C., Yuan, Q., Ye, L. and Zhou, X., **2018**. Substrate elasticity regulates adipose-derived stromal cell differentiation towards osteogenesis and adipogenesis through β-catenin transduction. *Acta Biomaterialia, 79*, pp.83–95.

53. Hefti, T., Frischherz, M., Spencer, N.D., Hall, H. and Schlottig, F., **2010**. A comparison of osteoclast resorption pits on bone with titanium and zirconia surfaces. *Biomaterials, 31*(28), pp.7321–7331.

54. Fu, W. and Zhang, W., **2017**. Hybrid AFM for nanoscale physicochemical characterization: Recent development and emerging applications. *Small, 13*(11), pp.1–16.

55. Bigerelle, M., Giljean, S. and Anselme, K., **2011**. Existence of a typical threshold in the response of human mesenchymal stem cells to a peak and valley topography. *Acta Biomaterialia, 7*(9), pp.3302–3311.

56. Dalby, M.J., Riehle, M.O., Sutherland, D.S., Agheli, H. and Curtis, A.S., **2004**. Use of nanotopography to study mechanotransduction in fibroblasts-methods and perspectives. *European Journal of Cell Biology, 283*(4), pp.159–169.

57. Jeon, H., Koo, S., Reese, W.M., Loskill, P., Grigoropoulos, C.P. and Healy, K.E., **2015**. Directing cell migration and organization via nanocrater-patterned cell-repellent interfaces. *Nature Materials, 14*(9), pp.918–923.

58. Dalby, M.J., Gadegaard, N., Tare, R., Andar, A., Riehle, M.O., Herzyk, P., Wilkinson, C.D. and Oreffo, R.O., **2007**. The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. *Nature Materials, 6*(12), pp.997–1003.

59. Pelipenko, J., Kocbek, P., Govedarica, B., Rošic, R., Baumgartner, S. and Kristl, J., **2013**. The topography of electrospun nanofibers and its impact on the growth and mobility of keratinocytes. *European Journal of Pharmaceutics and Biopharmaceutics, 84*(2), pp.401–411.

60. Pelipenko, J., Kocbek, P. and Kristl, J., **2015**. Critical attributes of nanofibers: Preparation, drug loading, and tissue regeneration. *International Journal of Pharmaceutics, 484*(1–2), pp.57–74.

61. Bottino, M.C., Thomas, V. and Janowski, G.M., **2011**. A novel spatially designed and functionally graded electrospun membrane for periodontal regeneration. *Acta Biomaterialia, 7*(1), pp.216–224.

62. Giannini, M., Primerano, C., Berger, L., Giannaccini, M., Wang, Z., Landi, E., Cuschieri, A., Dente, L., Signore, G. and Raffa, V., **2018**. Nano-topography: Quicksand for cell cycle progression? *Nanomedicine, 14*(8), pp.2656–2665.

63. Zouani, O.F., Chanseau, C., Brouillaud, B., Bareille, R., Deliane, F., Foulc, M.P., Mehdi, A. and Durrieu, M.C., **2012**. Altered nanofeature size dictates stem cell differentiation. *Journal of Cell Science, 125*(Pt. 5), pp.1217–1224.

64. Krishna, L., Dhamodaran, K., Jayadev, C., Chatterjee, K., Shetty, R., Khora, S.S. and Das, D., **2016**. Nanostructured scaffold as a determinant of stem cell fate. *Stem Cell Research & Therapy, 7*(1), pp.1–12.

65. Boyan, B.D., Lotz, E.M. and Schwartz, Z., **2017**. Roughness and hydrophilicity as osteogenic biomimetic surface properties. *Tissue Engineering Part, 23*(23–24), pp.1479–1489.

66. Chen, Z., Ni, S., Han, S., Crawford, R., Lu, S., Wei, F., Chang, J., Wu, C. and Xiao, Y., **2017**. Nanoporous microstructures mediate osteogenesis by modulating the osteo-immune response of macrophages. *Nanoscale, 9*(2), pp.706–718.

67. Kilian, K.A., Bugarija, B., Lahn, B.T. and Mrksich, M., **2010**. Geometric cues for directing the differentiation of mesenchymal stem cells. *Proceedings of the National Academy of Sciences of the United States of America, 107*(11), pp.4872–4827.

68. Higuchi, A., Ling, Q.D., Chang, Y., Hsu, S.T. and Umezawa, A., **2013**. Physical cues of biomaterials guide stem cell differentiation fate. *Chemical Reviews, 113*(5), pp.3297–3328.

69. Beckwith, K.S., Ullmann, S., Vinje, J. and Sikorski, P., **2019**. Influence of nanopillar arrays on fibroblast motility, adhesion, and migration mechanisms. *Small, 15*(43), p.e1902514.

70. Zhu, Y., Zhang, Q., Shi, X. and Han, D., **2019**. Hierarchical hydrogel composite interfaces with robust mechanical properties for biomedical applications. *Advanced Materials, 31*(45), p.e1804950.

71. Yin, M.J., Yao, M., Gao, S., Zhang, A.P., Tam, H.Y. and Wai, P.K., **2016**. Rapid 3D patterning of poly(acrylic acid) ionic hydrogel for miniature pH sensors. *Advanced Materials, 28*(7), pp.1394–1399.

72. Prasopthum, A., Cooper, M., Shakesheff, K.M. and Yang, J., **2019**. Three-dimensional printed scaffolds with controlled micro-/nanoporous surface topography direct chondrogenic and osteogenic differentiation of mesenchymal stem cells. *ACS Applied Materials & Interfaces, 11*(21), pp.18896–18906.

73. Jin, S.S., He, D.Q., Luo, D., Wang, Y., Yu, M., Guan, B., Fu, Y., Li, Z.X., Zhang, T., Zhou, Y.H., Wang, C.Y. and Liu, Y., **2019**. A biomimetic hierarchical nanointerface orchestrates macrophage polarization and mesenchymal stem cell recruitment to promote endogenous bone regeneration. *ACS Nano, 13*(6), pp.6581–6595.

74. Winklbauer, R., **2019**. Dynamic cell–cell adhesion mediated by pericellular matrix interaction-a hypothesis. *Journal of Cell Science, 132*(16), p.jcs231597.

75. Bershadsky, A., Kozlov, M. and Geiger, B., **2006**. Adhesion-mediated mechanosensitivity: A time to experiment, and a time to theorize. *Current Opinion in Cell Biology, 18*(5), pp.472–481.

76. Yang, J., Bai, R., Chen, B. and Suo, Z., **2020**. Hydrogel adhesion: A supramolecular synergy of chemistry, topology, and mechanics. *Advanced Functional Materials, 30*(2), pp.1–27.

77. Hynes, R.O., **2009**. The extracellular matrix: Not just pretty fibrils. *Science, 326*(5957), pp.1216–1219.

78. Hynes, R.O., **2002**. Integrins: Bidirectional, allosteric signaling machines. *Cell, 110*(6), pp.673–687.

79. Gonzalez-Fernandez, T., Sikorski, P. and Leach, J.K., **2019**. Bioinstructive materials for musculoskeletal regeneration. *Acta Biomaterialia, 96*, pp.20–34.

80. Park, M.H., Subbiah, R., Kwon, M.J., Kim, W.J., Kim, S.H., Park, K. and Lee, K., **2018**. The three dimensional cues-integrated-biomaterial potentiates differentiation of human mesenchymal stem cells. *Carbohydrate Polymers, 202*, pp.488–496.

81. Petersen, S., Alonso, J.M., Specht, A., Duodu, P., Goeldner, M. and del Campo, A., **2008**. Phototriggering of cell adhesion by caged cyclic RGD peptides. *Angewandte Chemie International Edition, 47*(17), pp.3192–3195.

82. Lock, J.G., Baschieri, F., Jones, M.C., Humphries, J.D., Montagnac, G., Strömblad, S. and Humphries, M.J., **2019**. Clathrin-containing adhesion complexes. *Journal of Cell Biology, 218*(7), pp.2086–2095.

83. Ouyang, L., Dan, Y., Shao, Z., Yang, S., Yang, C., Liu, G. and Duan, D., **2019**. MMP-sensitive PEG hydrogel modified with RGD promotes bFGF, VEGF and EPC-mediated angiogenesis. *Experimental and Therapeutic Medicine, 18*(4), pp.2933–2941.

84. Qu, C., Bao, Z., Zhang, X., Wang, Z., Ren, J., Zhou, Z., Tian, M., Cheng, X., Chen, X. and Feng, C., **2019**. A thermosensitive RGD-modified hydroxybutyl chitosan hydrogel as a 3D scaffold for BMSCs culture on keloid treatment. *International Journal of Biological Macromolecules, 125*, pp.78–86.

85. Kim, S., Cui, Z.K., Fan, J., Fartash. A., Aghaloo, T.L. and Lee, M., **2016**. Photocrosslinkable chitosan hydrogels functionalized with the RGD peptide and phosphoserine to enhance osteogenesis. *Journal of Materials Chemistry B, 4*(31), pp.5289–5298.

86. Lee, T.T., García, J.R., Paez, J.I., Singh, A., Phelps, E.A., Weis, S., Shafiq, Z., Shekaran, A., Del Campo, A. and García, A.J., **2015**. Light-triggered in vivo activation of adhesive peptides regulates cell adhesion, inflammation and vascularization of biomaterials. *Nature Materials, 14*(3), pp.352–360.

87. Zhang, Y., Lai, B.S. and Juhas, M., **2019**. Recent advances in aptamer discovery and applications. *Molecules, 24*(5), p.941.

88. Zhang, X., Battig, M.R., Chen, N., Gaddes, E.R., Duncan, K.L. and Wang, Y., **2016**. Chimeric aptamer-gelatin hydrogels as an extracellular matrix mimic for loading cells and growth factors. *Biomacromolecules, 17*(3), pp.778–787.

89. Zhao, N., Battig, M.R., Xu, M., Wang, X., Xiong, N. and Wang, Y., **2017**. Development of a dual-functional hydrogel using RGD and anti-VEGF aptamer. *Macromolecular Bioscience, 17*(11), p.1700201.

90. Chen, K., Feng, Y., Zhang, Y., Yu, L., Hao, X., Shao, F., Dou, Z., An, C., Zhuang, Z., Luo, Y., Wang, Y., Wu, J., Ji, P., Chen, T. and Wang, H., **2019**. Entanglement-driven adhesion, self-healing, and high stretchability of double-network PEG-based hydrogels. *ACS Applied Materials & Interfaces, 11*(40), pp.36458–36468.

91. Xu, J., Liu, Y. and Hsu, S.H., **2019**. Hydrogels based on schiff base linkages for biomedical applications. *Molecules, 24*(16), pp.1–21.

92. Quan, W.Y., Hu, Z., Liu, H.Z., Ouyang, Q.Q., Zhang, D.Y., Li, S.D., Li, P.W. and Yang, Z.M., **2019**. Mussel-inspired catechol-functionalized hydrogels and their medical applications. *Molecules, 24*(14), pp.1–27.

93. Han, L., Lu, X., Liu, K., Wang, K., Fang, L., Weng, L.T., Zhang, H., Tang, Y., Ren, F., Zhao, C., Sun, G., Liang, R. and Li, Z., **2017**. Mussel-inspired adhesive and tough hydrogel based on nanoclay confined dopamine polymerization. *ACS Nano, 11*(3), pp.2561–2574.

94. Gan, D., Xu, T., Xing, W., Ge, X., Fang, L., Wang, K., Ren, F. and Lu, X., **2019**. Mussel-inspired contact-active antibacterial hydrogel with high cell affinity, toughness, and recoverability. *Advanced Functional Materials, 29*(1), p.1805964.

95. Kim, B.J., Oh, D.X., Kim, S., Seo, J.H., Hwang, D.S., Masic, A., Han, D.K. and Cha, H.J., **2004**. Mussel-mimetic protein-based adhesive hydrogel. *Biomacromolecules, 15*(5), pp.1579–1585.

96. Liu, C., Wu, J., Gan, D., Li, Z., Shen, J., Tang, P., Luo, S., Li, P., Lu, X. and Zheng, W., **2020**. The characteristics of mussel-inspired nHA/OSA injectable hydrogel and repaired bone defect in rabbit. *Journal of Biomedical Materials Research Part B-Applied Biomaterials, 108*(5), pp.1814–1825.

97. Xie, C., Sun, W., Lu, H., Kretzschmann, A., Liu, J., Wagner, M., Butt, H.J. and Deng, X. and Wu, S., **2018**. Reconfiguring surface functions using visible-light-controlled metal-ligand coordination. *Nature Communications, 9*(1), pp.1–9.

98. Guo, Q., Chen, J., Wang, J., Zeng, H. and Yu, J., **2020**. Recent progress in synthesis and application of mussel-inspired adhesives. *Nanoscale, 12*(3), pp.1307–1324.

99. Zhou, T., Yan, L., Xie, C., Li, P., Jiang, L., Fang, J., Zhao, C., Ren, F., Wang, K., Wang, Y., Zhang, H., Guo, T. and Lu, X., **2019**. A mussel-inspired persistent ros-scavenging, electroactive, and osteoinductive scaffold based on electrochemical-driven in situ nanoassembly. *Small, 15*(25), pp.1–15.

100. Sun, W., Xue, B., Li, Y., Qin, M., Wu, J., Lu, K., Wu, J., Cao, Y., Jing, Q. and Wang, W., **2016**. Polymer-supramolecular polymer double-network hydrogel. *Advanced Functional Materials, 26*(48), pp.9044–9052.

101. Zazakowny, K., Lewandowska-Łańcucka, J., Mastalska-Popławska, J., Kamiński, K., Kusior, A., Radecka, M. and Nowakowska, M., **2016**. Biopolymeric hydrogels-nanostructured TiO_2 hybrid materials as potential injectable scaffolds for bone regeneration. *Colloids and Surfaces B: Biointerfaces, 148*, pp.607–614.

102. Oyen, M.L., **2014**. Mechanical characterisation of hydrogel materials. *International Materials Reviews, 59*(1), pp.44–59.

103. Callister, W.D. and Rethwisch, D.G., **2011**. *Materials Science and Engineering*. New York, John Wiley & Sons.

104. Ebenstein, D.M. and Pruitt, L.A., **2004**. Nanoindentation of soft hydrated materials for application to vascular tissues. *Journal of Biomedical Materials Research Part A, 69*(2), pp.222–232.

105. Loessner, D., Stok, K.S., Lutolf, M.P., Hutmacher, D.W., Clements, J.A. and Rizzi, S.C., **2010**. Bioengineered 3D platform to explore cell-ECM interactions and drug resistance of epithelial ovarian cancer cells. *Biomaterials, 31*(32), pp.8494–8506.

106. Wong, H.M., Zhao, Y., Tam, V., Wu, S., Chu, P.K., Zheng, Y., To, M.K., Leung, F.K., Luk, K.D., Cheung, K.M. and Yeung, K.W., **2013**. In vivo stimulation of bone formation by aluminum and oxygen plasma surface-modified magnesium implants. *Biomaterials, 34*(38), pp.9863–9876.

107. Engler, A.J., Sen, S., Sweeney, H.L. and Discher, D.E., **2006**. Matrix elasticity directs stem cell lineage specification. *Cell, 126*(4), pp.677–689.

108. Park, J., Kim, D.H. and Levchenko, A., **2018**. Topotaxis: A new mechanism of directed cell migration in topographic ECM gradients. *Biophysical Journal, 114*(6), pp.1257–1263.

109. Liao, I.C., Moutos, F.T., Estes, B.T., Zhao, X. and Guilak, F., **2013**. Composite three-dimensional woven scaffolds with interpenetrating network hydrogels to create functional synthetic articular cartilage. *Advanced Functional Materials, 23*(47), pp.5833–5839.

110. Shih, Y.R., Tseng, K.F., Lai, H.Y., Lin, C.H. and Lee, O.K., **2011**. Matrix stiffness regulation of integrin-mediated mechanotransduction during osteogenic differentiation of human mesenchymal stem cells. *Journal of Bone and Mineral Research, 26*(4), pp.730–738.

111. Dupont, S., Morsut, L., Aragona, M., Enzo, E., Giulitti, S., Cordenonsi, M., Zanconato, F., Le Digabel, J., Forcato, M., Bicciato, S., Elvassore, N. and Piccolo, S., **2011**. Role of YAP/TAZ in mechanotransduction. *Nature, 474*(7350), pp.179–183.

112. Obregon-Miano, F., Fathi, A., Rathsam, C., Sandoval, I., Deheghani, F. and Spahr, A., **2020**. Injectable porcine bone demineralized and digested extracellular matrix-PEGDA hydrogel blend for bone regeneration. *Journal of Materials Science—Materials in Medicine, 31*(2), p.21.

113. Okimura, C., Sakumura, Y., Shimabukuro, K. and Iwadate, Y., **2018**. Sensing of substratum rigidity and directional migration by fast-crawling cells. *Physical Review E, 97*(5), p.052401.

114. Raab, M., Swift, J., Dingal, P.C., Shah, P., Shin, J.W. and Discher, D.E., **2012**. Crawling from soft to stiff matrix polarizes the cytoskeleton and phosphoregulates myosin-II heavy chain. *Journal of Cell Biology, 199*(4), pp.669–683.

115. Lo, C.M., Wang, H.B., Dembo, M. and Wang, Y.L., **2000**. Cell movement is guided by the rigidity of the substrate. *Biophysical Journal, 79*(1), pp.144–152.

116. Vincent, L.G., Choi, Y.S., Alonso-Latorre, B., del Álamo, J.C. and Engler, A.J., **2013**. Mesenchymal stem cell durotaxis depends on substrate stiffness gradient strength. *Biotechnology Journal, 8*(4), pp.472–484.

117. Isenberg, B.C., Dimilla, P.A., Walker, M., Kim, S. and Wong, J.Y., **2009**. Vascular smooth muscle cell durotaxis depends on substrate stiffness gradient strength. *Biophysical Journal, 97*(5), pp.1313–1322.

118. Deng, Y., Zhao, X., Zhou, Y., Zhu, P., Zhang, L. and Wei, S., **2013**. In vitro growth of bioactive nanostructured apatites via agar-gelatin

hybrid hydrogel. *Journal of Biomedical Nanotechnology, 9*(12), pp.1972–1983.

119. Chang, A., Babhadiashar, N., Barrett-Catton, E. and Asuri, P., **2020**. Role of nanoparticle-polymer interactions on the development of double-network hydrogel nanocomposites with high mechanical strength. *Polymers (Basel), 12*(2), p.470.

120. Liao, J., Wang, B., Huang, Y., Qu, Y., Peng, J. and Qian, Z., **2017**. Injectable alginate hydrogel cross-linked by calcium gluconate-loaded porous microspheres for cartilage tissue engineering. *ACS Omega, 2*(2), pp.443–454.

121. Chen, Y., Zheng, K., Niu, L., Zhang, Y., Liu, Y., Wang, C. and Chu, F., **2019**. Highly mechanical properties nanocomposite hydrogels with biorenewable lignin nanoparticles. *International Journal of Biological Macromolecules, 128*, pp.414–420.

122. Zhai, X., Hou, C., Pan, H., Lu, W.W., Liu, W. and Ruan, C., **2018**. Nanoclay incorporated polyethylene-glycol nanocomposite hydrogels for stimulating in vitro and in vivo osteogenesis. *Journal of Biomedical Nanotechnology, 14*(4), pp.662–674.

123. Xu, Y., Wu, X., Wang, S., Yang, C., Li, Y. and Cao, Y., **2019**. Hydroxyapatite nanoparticle-crosslinked peptide hydrogels for three-dimensional culture and differentiation of MC3T3-E1 osteoblasts. *Journal of Biomedical Nanotechnology, 15*(12), pp.2351–2362.

124. Zhao, Q., Chen, Y. and Liu, Y., **2018**. A polysaccharide/tetraphenylethylene-mediated blue-light emissive and injectable supramolecular hydrogel. *Chinese Chemical Letters, 29*(1), pp.84–86.

125. Gao, Y. and Dong, C.M., **2018**. Quadruple thermo-photo-redox-responsive random copolypeptide nanogel and hydrogel. *Chinese Chemical Letters, 29*(6), pp.927–930.

126. Liu, B., Wang, Y., Miao, Y., Zhang, X., Fan, Z., Singh, G., Zhang, X., Xu, K., Li, B., Hu, Z. and Xing, M., **2018**. Hydrogen bonds autonomously powered gelatin methacrylate hydrogels with super-elasticity, self-heal and underwater self-adhesion for sutureless skin and stomach surgery and E-skin. *Biomaterials, 171*, pp.83–96.

127. Zhang, J., Chen, L., Shen, B., Chen, L. and Feng, J., **2019**. Ultra-high strength poly(N-(2-hydroxyethyl) acrylamide)/chitosan hydrogel with "repelling and killing" bacteria property. *Carbohydrate Polymers, 225*, p.115160.

128. Wang, Y., Xue, Y., Wang, J., Zhu, Y., Zhu, Y., Zhang, X., Liao, J., Li, X., Wu, X., Qin, Y.X. and Chen, W., **2019**. A composite hydrogel with high mechanical strength, fluorescence, and degradable behavior for bone tissue engineering. *Polymers (Basel), 11*(7), p.1112.

129. Haul, R.S.J. and Gregg, K.S.W., **1982**. Sing: Adsorption, surface area and porosity. 2. Auflage, Academic Press, London 1982. 303 Seiten, Preis: $ 49.50. *Berichte der Bunsengesellschaft für physikalische Chemie, 86*(10), p.957.

130. Menger, F.M. and Bonicamp, J.M. **1981**. Experimental test for micelle porosity. *Journal of the American Chemical Society, 103*(8), pp.2140–2141.

131. Spiller, K.L., Laurencin, S.J., Charlton, D., Maher, S.A. and Lowman, A.M., **2008**. Superporous hydrogels for cartilage repair: Evaluation of the morphological and mechanical properties. *Acta Biomaterialia, 4*(1), pp.17–25.

132. Ziel, R., Haus, A. and Tulke, A., **2008**. Quantification of the pore size distribution (porosity profiles) in microfiltration membranes by SEM, TEM and computer image analysis. *Journal of Membrane Science, 323*(2), pp.241–246.

133. Gao, Y., Li, B., Kong, W., Yuan, L., Guo, L., Li, C., Fan, H., Fan, Y. and Zhang, X., **2018**. Injectable and self-crosslinkable hydrogels based on collagen type II and activated chondroitin sulfate for cell delivery. *International Journal of Biological Macromolecules, 118*(Pt. B), pp.2014–2020.

134. Li, M., Mondrinos, M.J., Chen, X., Gandhi, M.R., Ko, F.K. and Lelkes, P.I., **2006**. Co-electrospun poly(lactide-co-glycolide), gelatin, and elastin blends for tissue engineering scaffolds. *Journal of Biomedical Materials Research Part A, 79*(4), pp.963–973.

135. Lien, S.M., Ko, L.Y. and Huang, T.J., **2009**. Effect of pore size on ECM secretion and cell growth in gelatin scaffold for articular cartilage tissue engineering. *Acta Biomaterialia, 5*(2), pp.670–679.

136. Matsiko, A., Gleeson, J.P. and O'Brien, F.J., **2015**. Scaffold mean pore size influences mesenchymal stem cell chondrogenic differentiation and matrix deposition. *Tissue Engineering Part, 21*(3–4), pp.486–497.

137. Zhao, Y., Tan, K., Zhou, Y., Ye, Z. and Tan, W.S., **2016**. A combinatorial variation in surface chemistry and pore size of three-dimensional porous poly(ε-caprolactone) scaffolds modulates the behaviors of mesenchymal stem cells. *Materials Science & Engineering C-Materials for Biological Applications, 59*, pp.193–202.

138. Gao, X., Gao, L., Groth, T., Liu, T., He, D., Wang, M., Gong, F., Chu, J. and Zhao, M., **2019**. Fabrication and properties of an injectable sodium alginate/PRP composite hydrogel as a potential cell carrier for cartilage repair. *Journal of Biomedical Materials Research Part A, 107*(9), pp.2076–2087.

139. Chang, K.H., Liao, H.T. and Chen, J.P., **2013**. Preparation and characterization of gelatin/hyaluronic acid cryogels for adipose tissue engineering: In vitro and in vivo studies. *Acta Biomaterialia, 9*(11), pp.9012–9026.

140. Peng, L., Zhou, Y., Lu, W., Zhu, W., Li, Y., Chen, K., Zhang, G., Xu, J., Deng, Z. and Wang, D., **2019**. Characterization of a novel polyvinyl alcohol/chitosan porous hydrogel combined with bone marrow mesenchymal stem cells and its application in articular cartilage repair. *BMC Musculoskeletal Disorders, 20*(1), pp.1–12.

141. Suneetha, M., Rao, K.M. and Han, S.S., **2020**. Mechanically improved porous hydrogels with polysaccharides via polyelectrolyte complexation for bone tissue engineering. *International Journal of Biological Macromolecules, 144*, pp.160–169.

142. Gu, L., Zhang, J., Li, L., Du, Z., Cai, Q. and Yang, X., **2019**. Hydroxyapatite nanowire composited gelatin cryogel with improved mechanical properties and cell migration for bone regeneration. *Biomedical Materials, 14*(4), p.045001.

143. Hwang, C.M., Sant, S., Masaeli, M., Kachouie, N.N., Zamanian, B., Lee, S.H. and Khademhosseini, A., **2010**. Fabrication of three-dimensional porous cell-laden hydrogel for tissue engineering. *Biofabrication, 2*(3), p.035003.

144. Grenier, J., Duval, H., Barou, F., Lv, P., David, B. and Letourneur, D., **2019**. Mechanisms of pore formation in hydrogel scaffolds textured by freeze-drying. *Acta Biomaterialia, 94*, pp.195–203.

145. Sharma, S., Panitch, A. and Neu, C.P., **2013**. Incorporation of an aggrecan mimic prevents proteolytic degradation of anisotropic cartilage analogs. *Acta Biomaterialia, 9*(1), pp.4618–4625.

146. Namazi, H., Hasani, M. and Yadollahi, M., **2019**. Antibacterial oxidized starch/ZnO nanocomposite hydrogel: Synthesis and evaluation of its swelling behaviours in various pHs and salt solutions. *International Journal of Biological Macromolecules, 126*, pp.578–584.

147. Gilarska, A., Lewandowska-Łańcucka, J., Guzdek-Zając, K., Karewicz, A., Horak, W., Lach, R., Wójcik, K. and Nowakowska, M., **2020**. Bioactive yet antimicrobial structurally stable collagen/chitosan/lysine functionalized hyaluronic acid-based injectable hydrogels for potential bone tissue engineering applications. *International Journal of Biological Macromolecules, 155*, pp.938–950.

148. Xu, C., Su, P., Chen, X., Meng, Y., Yu, W., Xiang, A.P. and Wang, Y., **2011**. Biocompatibility and osteogenesis of biomimetic bioglass-collagen-phosphatidylserine composite scaffolds for bone tissue engineering. *Biomaterials, 32*(4), pp.1051–1058.

149. Karageorgiou, V. and Kaplan, D., **2005**. Porosity of 3D biomaterial scaffolds and osteogenesis. *Biomaterials, 26*(27), pp.5474–5491.

150. Kostina, N.Y., Blanquer, S., Pop-Georgievski, O., Rahimi, K., Dittrich, B., Höcherl, A., Michálek, J., Grijpma, D.W. and

Rodriguez-Emmenegger, C., **2019**. Zwitterionic functionalizable scaffolds with gyroid pore architecture for tissue engineering. *Macromolecular Bioscience, 19*(4), pp.1–9.

151. Hjelle, K., Solheim, E., Strand, T., Muri, R. and Brittberg, M., **2002**. Articular cartilage defects in 1,000 knee arthroscopies. *Arthroscopy, 18*(7), pp.730–734.

152. Dey, K., Agnelli, S., Re, F., Russo, D., Lisignoli, G., Manferdini, C., Bernardi, S., Gabusi, E. and Sartore, L., **2019**. Rational design and development of anisotropic and mechanically strong gelatin-based stress relaxing hydrogels for osteogenic/chondrogenic differentiation. *Macromolecular Bioscience, 19*(8), pp.1–18.

153. Taqvi, S., Dixit, L. and Roy, K., **2006**. Biomaterial-based notch signaling for the differentiation of hematopoietic stem cells into T cells. *Journal of Biomedical Materials Research Part A, 79*(3), pp.689–697.

154. Villanueva. I., Klement, B.J., von Deutsch, D. and Bryant, S.J., **2009**. Cross-linking density alters early metabolic activities in chondrocytes encapsulated in poly(ethylene glycol) hydrogels and cultured in the rotating wall vessel. *Biotechnology and Bioengineering, 102*(4), pp.1242–1250.

155. Bryant, S.J., Chowdhury, T.T., Lee, D.A., Bader, D.L. and Anseth, K.S., **2004**. Crosslinking density influences chondrocyte metabolism in dynamically loaded photocrosslinked poly(ethylene glycol) hydrogels. *Annals of Biomedical Engineering, 32*(3), pp.407–417.

156. Al-Sabah, A., Burnell, S.E.A., Simoes, I.N., Jessop, Z., Badiei, N., Blain, E. and Whitaker, I.S., **2019**. Structural and mechanical characterization of crosslinked and sterilised nanocellulose-based hydrogels for cartilage tissue engineering. *Carbohydrate Polymers, 212*, pp.242–251.

157. Liao, J., Qu, Y., Chu, B., Zhang, X. and Qian, Z., **2015**. Biodegradable CSMA/PECA/graphene porous hybrid scaffold for cartilage tissue engineering. *Scientific Reports, 5*, pp.1–16.

158. Wang, X., Xu, S., Zhou, S., Xu, W., Leary, M., Choong, P., Qian, M., Brandt, M. and Xie, Y.M., **2016**. Topological design and additive manufacturing of porous metals for bone scaffolds and orthopaedic implants: A review. *Biomaterials, 83*, pp.127–141.

159. Wegst, U.G., Bai, H., Saiz, E., Tomsia, A.P. and Ritchie, R.O., **2015**. Bioinspired structural materials. *Nature Materials, 14*(1), pp.23–36.

160. Phadke, A., Hwang, Y., Kim, S.H., Kim, S.H., Yamaguchi, T., Masuda, K. and Varghese, S., **2013**. Effect of scaffold microarchitecture on osteogenic differentiation of human mesenchymal stem cells. *European Cells and Materials, 25*, pp.114–129.

161. Bao, M., Xie, J., Piruska, A. and Huck, W.T.S., **2017**. 3D microniches reveal the importance of cell size and shape. *Nature Communications, 8*(1), pp.1–12.

162. Di Luca, A., Lorenzo-Moldero, I., Mota, C., Lepedda, A., Auhl, D., Van Blitterswijk, C. and Moroni, L., **2016**. Tuning cell differentiation into a 3D scaffold presenting a pore shape gradient for osteochondral regeneration. *Advanced Healthcare Materials, 5*(14), pp.1753–1763.

163. Ferlin, K.M., Prendergast, M.E., Miller, M.L., Kaplan, D.S. and Fisher, J.P., **2016**. Influence of 3D printed porous architecture on mesenchymal stem cell enrichment and differentiation. *Acta Biomaterialia, 32*, pp.161–169.

164. Lu, L., Yuan, S., Wang, J., Shen, Y., Deng, S., Xie, L. and Yang, Q., **2018**. The formation mechanism of hydrogels. *Current Stem Cell Research & Therapy, 13*(7), pp.490–496.

165. Zustiak, S.P. and Leach, J.B., **2010**. Hydrolytically degradable poly(ethylene glycol) hydrogel scaffolds with tunable degradation and mechanical properties. *Biomacromolecules, 11*(5), pp.1348–1357.

166. Cha, C., Kohman, R.E. and Kong, H. **2009**. Biodegradable polymer crosslinker: Independent control of stiffness, toughness, and hydrogel degradation rate. *Advanced Functional Materials, 19*(19), pp.3056–3062.

167. Kharkar, P.M., Kiick, K.L. and Kloxin, A.M., **2013**. Designing degradable hydrogels for orthogonal control of cell microenvironments. *Chemical Society Reviews, 42*(17), pp.7335–7372.

168. Bitter, T. and Muir, H.M., **1962**. A modified uronic acid carbazole reaction. *Analytical Biochemistry, 4*, pp.330–334.

169. Benoit, D.S., Durney, A.R. and Anseth, K.S., **2006**. Manipulations in hydrogel degradation behavior enhance osteoblast function and mineralized tissue formation. *Tissue Engineering, 12*(6), pp.1663–1673.

170. Kong, H.J., Alsberg, E., Kaigler, D., Lee, K.Y. and Mooney, D.J., **2004**. Controlling degradation of hydrogels via the size of crosslinked junctions. *Advanced Materials, 16*(21), pp.1917–1921.

171. Kretlow, J.D. and Mikos, A.G., **2007**. AIChE Alpha Chi Sigma Award: From material to tissue: Biomaterial development, scaffold fabrication, and tissue engineering. *AIChE Journal, 54*(12), pp.3048–3067.

172. Cui, N., Qian, J., Liu, T., Zhao, N. and Wang, H., **2015**. Hyaluronic acid hydrogel scaffolds with a triple degradation behavior for bone tissue engineering. *Carbohydrate Polymers, 126*, pp.192–198.

173. Holloway, J.L., Ma, H., Rai, R. and Burdick, J.A., **2014**. Modulating hydrogel crosslink density and degradation to control bone morphogenetic protein delivery and in vivo bone formation. *Journal of Controlled Release, 191*, pp.63–70.

174. Kim, S., Cui, Z.K., Koo, B., Zheng, J., Aghaloo, T. and Lee, M., **2018**. Chitosan-lysozyme conjugates for enzyme-triggered hydrogel degradation in tissue engineering applications. *ACS Applied Materials & Interfaces, 10*(48), pp.41138–41145.

175. Kunjukunju, S., Roy, A., Shekhar, S. and Kumta, P.N., **2018**. Crosslinked enzyme aggregates of alginate lyase: A systematic engineered approach to controlled degradation of alginate hydrogel. *International Journal of Biological Macromolecules, 115*, pp.176–184.

176. Pradhan, S., Keller, K.A., Sperduto, J.L. and Slater, J.H., **2017**. Fundamentals of laser-based hydrogel degradation and applications in cell and tissue engineering. *Advanced Healthcare Materials, 6*(24), p.1700681.

177. Brandenberg, N. and Lutolf, M.P., **2016**. In situ patterning of microfluidic networks in 3D cell-laden hydrogels. *Advanced Materials, 28*(34), pp.7450–7456.

178. Hu, J., Chen, Y., Li, Y., Zhou, Z. and Cheng, Y., **2017**. A thermo-degradable hydrogel with light-tunable degradation and drug release. *Biomaterials, 112*, pp.133–140.

179. Pina, S., Oliveira, J.M. and Reis, R.L., **2015**. Natural-based nanocomposites for bone tissue engineering and regenerative medicine: A review. *Advanced Materials, 27*(7), pp.1143–1169.

180. Cukierman, E., Pankov, R., Stevens, D.R. and Yamada, K.M., **2001**. Taking cell-matrix adhesions to the third dimension. *Science, 294*(5547), pp.1708–1712.

181. Balcioglu, H.E., Balasubramaniam, L., Stirbat, T.V., Doss, B.L., Fardin, M.A., Mège, R.M. and Ladoux, B., **2020**. A subtle relationship between substrate stiffness and collective migration of cell clusters. *Soft Matter, 16*(7), pp.1825–1839.

182. Zou, C., Luo, Q., Qin, J., Shi, Y., Yang, L., Ju, B. and Song, G., **2013**. Osteopontin promotes mesenchymal stem cell migration and lessens cell stiffness via integrin β1, FAK, and ERK pathways. *Cell Biochemistry and Biophysics, 65*(3), pp.455–462.

183. Hammond, J.W., Cai, D. and Verhey, K.J., **2008**. Tubulin modifications and their cellular functions. *Current Opinion in Cell Biology, 20*(1), pp.71–76.

184. Hesse, E., Hefferan, T.E., Tarara, J.E., Haasper, C., Meller, R., Krettek, C., Lu, L. and Yaszemski, M.J., **2010**. Collagen type I hydrogel allows migration, proliferation, and osteogenic differentiation of rat bone marrow stromal cells. *Journal of Biomedical Materials Research Part A, 94*(2), pp.442–449.

185. Maisani, M., Ziane, S., Ehret, C., Levesque, L., Siadous, R., Le Meins, J.F., Chevallier, P., Barthélémy, P., De Oliveira, H., Amédée, J., Mantovani, D. and Chassande, O., **2018**. A new composite hydrogel combining the biological properties of collagen with the mechanical properties of a supramolecular scaffold for bone tissue engineering. *Journal of Tissue Engineering and Regenerative Medicine, 12*(3), pp.e1489–e1500.

186. Mredha, M.T.I., Kitamura, N., Nonoyama, T., Wada, S., Goto, K., Zhang, X., Nakajima, T., Kurokawa, T., Takagi, Y., Yasuda, K. and Gong, J.P., **2017**. Anisotropic tough double network hydrogel from fish collagen and its spontaneous in vivo bonding to bone. *Biomaterials, 132*, pp.85–95.

187. Duan, P. and Bonewald, L.F., **2016**. The role of the wnt/β-catenin signaling pathway in formation and maintenance of bone and teeth. *International Journal of Biochemistry and Cell Biology, 77*(Pt. A), pp.23–29.

188. Glass, D.A. and Karsenty, G., **2006**. Molecular bases of the regulation of bone remodeling by the canonical Wnt signaling pathway. *Current Topics in Developmental Biology, 73*, pp.43–84.

189. Arima, Y. and Iwata, H., **2007**. Effect of wettability and surface functional groups on protein adsorption and cell adhesion using well-defined mixed self-assembled monolayers. *Biomaterials, 28*(20), pp.3074–3082.

190. Park, H., Guo, X., Temenoff, J.S., Tabata, Y., Caplan, A.I., Kasper, F.K. and Mikos, A.G., **2009**. Effect of swelling ratio of injectable hydrogel composites on chondrogenic differentiation of encapsulated rabbit marrow mesenchymal stem cells in vitro. *Biomacromolecules, 10*(3), pp.541–546.

191. Xiao, X., Wang, W., Liu, D., Zhang, H., Gao, P., Geng, L., Yuan, Y., Lu, J. and Wang, Z., **2015**. The promotion of angiogenesis induced by three-dimensional porous beta-tricalcium phosphate scaffold with different interconnection sizes via activation of PI3K/Akt pathways. *Scientific Reports, 5*, pp.1–11.

192. Doyle, A.D., Carvajal, N., Jin, A., Matsumoto, K. and Yamada, K.M., **2015**. Local 3D matrix microenvironment regulates cell migration through spatiotemporal dynamics of contractility-dependent adhesions. *Nature Communications, 6*, pp.1–15.

193. Daly, A.C., Pitacco, P., Nulty, J., Cunniffe, G.M. and Kelly, D.J., **2018**. 3D printed microchannel networks to direct vascularisation during endochondral bone repair. *Biomaterials, 162*, pp.34–46.

194. Giammanco, G.E., Carrion, B., Coleman, R.M. and Ostrowski, A.D., **2016**. Photoresponsive polysaccharide-based hydrogels with tunable mechanical properties for cartilage tissue engineering. *ACS Applied Materials & Interfaces, 8*(23), pp.14423–14439.

195. Joeng, K.S. and Long, F., **2014**. Wnt7b can replace Ihh to induce hypertrophic cartilage vascularization but not osteoblast differentiation during endochondral bone development. *Bone Research*, *2*, p.14004.

196. Zhang, N., Lock, J., Sallee, A. and Liu, H., **2015**. Magnetic nanocomposite hydrogel for potential cartilage tissue engineering: Synthesis, characterization, and cytocompatibility with bone marrow derived mesenchymal stem cells. *ACS Applied Materials & Interfaces, 7*(37), pp.20987–20998.

197. Thoniyot, P., Tan, M.J., Karim, A.A., Young, D.J. and Loh, X.J., **2015**. Nanoparticle-hydrogel composites: Concept, design, and applications of these promising, multi-functional materials. *Advanced Science, 2*(1–2), p.1400010.

198. Gao, F., Xu, Z., Liang, Q., Li, H., Peng, L., Wu, M., Zhao, X., Cui, X., Ruan, C. and Liu, W., **2019**. Osteochondral regeneration with 3D-printed biodegradable high-strength supramolecular polymer reinforced-gelatin hydrogel scaffolds. *Advanced Science, 6*(15), p.1900867.

199. Kim, K. and Kim, M.S., **2016**. An injectable hydrogel derived from small intestine submucosa as a stem cell carrier. *Journal of Biomedical Materials Research Part B-Applied Biomaterials, 104*(8), pp.1544–1550.

200. Bajestani, M.I., Kader, S., Monavarian, M., Mousavi, S.M., Jabbari, E. and Jafari, A., **2020**. Material properties and cell compatibility of poly(γ-glutamic acid)-keratin hydrogels. *International Journal of Biological Macromolecules, 142*, pp.790–802.

201. McGregor, N.E., Murat, M., Elango, J., Poulton, I.J., Walker, E.C., Crimeen-Irwin, B., Ho, P.W.M., Gooi, J.H., Martin, T.J. and Sims, N.A., **2019**. IL-6 exhibits both cis- and trans-signaling in osteocytes and osteoblasts, but only trans-signaling promotes bone formation and osteoclastogenesis. *Journal of Biological Chemistry, 294*(19), pp.7850–7863.

202. Pal, P., Nguyen, Q.C., Benton, A.H., Marquart, M.E. and Janorkar, A.V., **2019**. Drug-Loaded elastin-like polypeptide-collagen hydrogels with high modulus for bone tissue engineering. *Macromolecular Bioscience, 19*(9), pp.1–13.

203. Fan, W., Zuo, L., Zhang, Y., Chen, Y. and Liu, T., **2018**. Mechanically strong polyimide/carbon nanotube composite aerogels with controllable porous structure. *Composites Science and Technology, 156*, pp.186–191.

204. Stagnaro, P., Schizzi, I., Utzeri, R., Marsano, E. and Castellano, M., **2018**. Alginate-polymethacrylate hybrid hydrogels for potential osteochondral tissue regeneration. *Carbohydrate Polymers, 185*, pp.56–62.

205. Nkhwa, S., Iskandar, L., Gurav, N. and Deb, S., **2018**. Combinatorial design of calcium meta phosphate poly(vinyl alcohol) bone-like biocomposites. *Journal of Materials Science—Materials in Medicine, 29*(8), p.128.

206. Dey, R.E., Wimpenny, I., Gough, J.E., Watts, D.C. and Budd, P.M., **2018**. Poly(vinylphosphonic acid-co-acrylic acid) hydrogels: The effect of copolymer composition on osteoblast adhesion and proliferation. *Journal of Biomedical Materials Research Part A, 106*(1), pp.255–264.

207. Medhat, D., Rodríguez, C.I. and Infante, A., **2019**. Immunomodulatory effects of MSCs in bone healing. *International Journal of Molecular Sciences, 20*(21), p.5467.

208. Huang, J., Chen, L., Gu, Z. and Wu, J., **2019**. Red jujube-incorporated gelatin methacryloyl (gelma) hydrogels with anti-oxidation and immunoregulation activity for wound healing. *Journal of Biomedical Nanotechnology, 15*(7), pp.1357–1370.

209. King, S.N., Hanson, S.E., Chen, X., Kim, J., Hematti, P. and Thibeault, S.L., **2014**. In vitro characterization of macrophage interaction with mesenchymal stromal cell-hyaluronan hydrogel constructs. *Journal of Biomedical Materials Research Part A, 102*(3), pp.890–902.

210. Nathan, K., Lu, L.Y., Lin, T., Pajarinen, J., Jämsen, E., Huang, J.F., Romero-Lopez, M., Maruyama, M., Kohno, Y., Yao, Z. and Goodman, S.B., **2019**. Precise immunomodulation of the M1 to M2 macrophage transition enhances mesenchymal stem cell osteogenesis and differs by sex. *Bone & Joint Research, 8*(10), pp.481–488.

211. McWhorter, F.Y., Wang, T., Nguyen, P., Chung, T. and Liu, W.F., **2013**. Modulation of macrophage phenotype by cell shape. *Proceedings of the National Academy of Sciences of the United States of America, 110*(43), pp.17253–17258.

212. Shi, M., Wang, C., Wang, Y., Tang, C., Miron, R.J. and Zhang, Y., **2018**. Deproteinized bovine bone matrix induces osteoblast differentiation via macrophage polarization. *Journal of Biomedical Materials Research Part A, 106*(5), pp.1236–1246.

213. Qiu, P., Li, M., Chen, K., Fang, B., Chen, P., Tang, Z., Lin, X. and Fan, S., **2020**. Periosteal matrix-derived hydrogel promotes bone repair through an early immune regulation coupled with enhanced angio- and osteogenesis. *Biomaterials, 227*, p.119552.

214. Luo, M.L., Jiao, Y., Gong, W.P., Li, Y., Niu, L.N., Tay, F.R. and Chen, J.H., **2020**. Macrophages enhance mesenchymal stem cell osteogenesis via down-regulation of reactive oxygen species. *Journal of Dentistry*, *94*, p.103297.

215. Rowley, A.T., Nagalla, R.R., Wang, S.W. and Liu, W.F., **2019**. Extracellular matrix-based strategies for immunomodulatory biomaterials engineering. *Advanced Healthcare Materials, 8*(8), p.e1801578.

216. Chen, P., Cescon, M., Zuccolotto, G., Nobbio, L., Colombelli, C., Filaferro, M., Vitale, G., Feltri, M.L. and Bonaldo, P., **2015**. Collagen VI regulates peripheral nerve regeneration by modulating macrophage recruitment and polarization. *Acta Neuropathologica, 129*(1), pp.97–113.

217. Tanaka, R., Saito, Y., Fujiwara, Y., Jo, J.I. and Tabata, Y., **2019**. Preparation of fibrin hydrogels to promote the recruitment of anti-inflammatory macrophages. *Acta Biomaterialia, 89*, pp.152–165.

218. Cascone, S. and Lamberti, G., **2020**. Hydrogel-based commercial products for biomedical applications: A review. *International Journal of Pharmaceutics, 573*, p.118803.

219. de Queiroz, A.A.B., Debieux, P., Amaro, J., Ferretti, M. and Cohen, M., **2018**. Hydrogel implant is as effective as osteochondral autologous transplantation for treating focal cartilage knee injury in 24 months. *Knee Surgery, Sports Traumatology, Arthroscopy, 26*(10), pp.2934–2941.