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Bioactive hydrogels for bone regeneration

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ABSTRACT

Bone self-healing is limited and generally requires external intervention to augment bone repair and regeneration. While traditional methods for repairing bone defects such as autografts, allografts, and xenografts have been widely used, they all have corresponding disadvantages, thus limiting their clinical use. Despite the development of a variety of biomaterials, including metal implants, calcium phosphate cements (CPC), hydroxyapatite, etc., the desired therapeutic effect is not fully achieved. Currently, polymeric scaffolds, particularly hydrogels, are of interest and their unique configurations and tunable physicochemical properties have been extensively studied. This review will focus on the applications of various cutting-edge bioactive hydrogels systems in bone regeneration, as well as their advantages and limitations. We will examine the composition and defects of the bone, discuss the current biomaterials for bone regeneration, and classify recently developed polymeric materials for hydrogel synthesis. We will also elaborate on the properties of desirable hydrogels as well as the fabrication techniques and different delivery strategies. Finally, the existing challenges, considerations, and the future prospective of hydrogels in bone regeneration will be outlined.

1. Introduction

In recent years, the medical cost of treating bone related trauma, infection, and tumor has continuously increased [1]. According to relative statistics, the market of European bone graft substitutes was $177 million in 2010, and the global market value of orthopedics biomaterials was $1.9 billion in the same year [2]. It is forecasted to reach $3.3 billion in 2017, presenting a huge expenditure to the national economy. Bone development is a dynamic process [3]. Various cytokines and growth factors recruit osteoprogenitors to the injury site, and subsequently guide them to differentiate into osteoblasts [4]. However, in the case of severe injuries or individuals with congenital malformation, osteogenesis imperfecta, rheumatoid arthritis, or osteoporosis, the lengthy and limited self-healing process cannot adequately satisfy the requirements of timely bone repair [5–7]. Thus, bone augmentation needs to be considered [8]. Current clinical treatments for bone injuries such as autografts, allografts, and xenografts failed to be used extensively due to potential risks of disease transmission, infection, and host rejection [9]. Bone tissue engineering (BTE), a novel approach using scaffolds seeding cells or incorporating bioactive growth factors to promote bone repair and regeneration, is believed to be able to avoid the aforementioned issues and provide an innovative platform in regenerative medicine [10]. The scaffolds used in bone tissue engineering aim at providing structural support, creating an appropriate environment for cell adhesion, migration, proliferation and differentiation, and recapitulating the functional activity of the bone defects [3].

Materials employed for scaffolds can be divided into inorganic materials, natural or synthetic polymers, and composite materials [11]. Numerous studies concerning inorganic materials used in bone repair have emerged. Ceramics are a kind of inorganic

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material that demonstrate good mechanical properties and osteoconductiveness, and have been successfully used in alveolar bone repair. In the past decades, polymer scaffolds have been widely investigated in bone tissue engineering. Commonly used natural materials, such as collagen and chitosan, are considered to be biodegradable and bio-absorbable, and synthetic polymers like poly (lactic acid) (PLA) and poly (D, L-lactide-co-glycolide) (PLGA) are thought to have tunable mechanical properties; however, the structure and characteristics of cells and native tissues are rarely considered in the design of polymeric scaffolds. Therefore, the scaffolds usually demonstrate poor integration with surrounding bone tissues [12]. New materials and solutions are continually developed to meet medical needs [13]. Recently, the interaction between scaffolds and native tissues, particularly the indispensable role of the natural extracellular matrix (ECM) in bone repair, has been extensively investigated. Due to their added advantages of biocompatibility and biodegradability over inorganic materials, polymer materials and composites have shown to integrate well with surrounding bone tissue, allowing a stabilized anchorage of implants and preventing an immune response.

Hydrogels, a type of polymer scaffold, have several potential advantages in bone repair. Hydrogels are composed of three-dimensional hydrophilic polymer chains, which have superior mechanical strength and can provide nutrient environments suitable for endogenous cell growth. They are able to mimic the natural ECM of the bone, thus presenting a prospective ability to encapsulate bioactive molecules or cells. Due to the network structure of the hydrogels, the entrapped proteins or cells are confined in the meshes and they hydrogels can control the release of the materials as required [13]. Moreover, hydrogels are absorbable and demonstrate excellent integration with surrounding tissues, thereby avoiding the complexity of surgical removal and reducing the possibility of an inflammatory response [14]. Additionally, raw materials for preparation of hydrogels are extensive and readily available, and they can be tailored to obtain the desired geometry for implantation or injection, and the degradation rate and porosity or release profile can be easily controlled by altering the cross-linking method and degree.

Nevertheless, challenges concerning the controlled release of encapsulated drugs, proteins or cells still need to be further investigated. Both the burst and delayed release of the drug can affect the actual therapeutic effect, and the use of inappropriate polymers can also cause toxic reactions.

This review will broaden our understanding of the design, development, and challenges of hydrogel-based bone regeneration. We will first briefly introduce the structure and composition of the bone, and then discuss the types of the bone defects and current available clinical treatments. As Fig. 1 illustrates, we will elaborate on the application of polymers and the revolutionary bioactive hydrogels developed for bone repair and regeneration by identifying the requirements of successful formulations and reporting the innovative modifications that overcome the fundamental challenges associated with hydrogels. The benefits and potential complications of delivery strategies in treating bone defects will be detailed and explored. Finally, the limitations of current developments and future directions for the development of hydrogel-based bone regeneration will be discussed.

2. Bone anatomy and current bone injury treatments

2.1. Composition and defects of bone tissues

Bone is a hard and dense tissue mainly composed of two parts, cortical bone and cancellous, or trabecular bone [15]. The ECM of the bone is a biphasic system, one third of which is composed of organic matter, predominantly type I collagen fibers, and the remaining two thirds consist of inorganic matter or bone salt, such as hydroxyapatite-like calcium phosphates. Three cell types – osteoblasts, osteocytes and osteoclasts – work in concordance to form a unified bone organism. Osteoblasts are the main functional

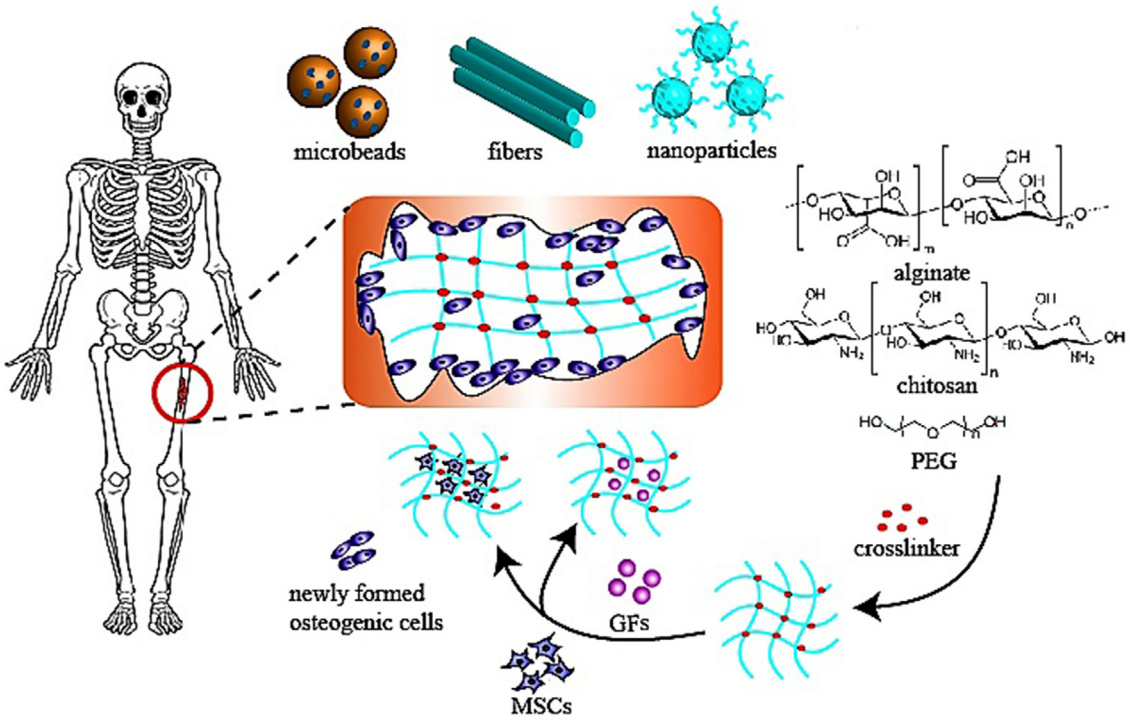

Fig. 1. Schematic illustration of hydrogel-assisted bone regeneration.

cells of bone formation and are responsible for the synthesis, secretion, and mineralization of bone matrix. They stem from mesenchymal stem cells (MSCs), or progenitor cells, from the adherent portion of bone marrow and cover the surface of bone seams, forming a protein-like mixture called osteoid, which mainly consists of polymerized collagen chains, and is later mineralized into bone, mediated by the deposition of calcium and phosphate. Additionally, osteoblasts also produce the corresponding hormones that promote surrounding bone formation. Osteocytes are inactive post-synthetic osteoblasts that migrated to the ECM matrix of bone. They connect with osteoblasts and other cells and play an important role in mineral homeostasis. The third type of cells, osteoclasts, are derived from hematopoietic stem cells in the non-adherent portion of marrow and are primarily responsible for bone resorption via secreting various matters. The coordinated interactions between osteoblasts and osteoclasts maintain the normal bone mass and aid in the final bone remodeling [16].

The majority of bone damage, defects, and injuries can be classified into the following categories: fractures, old age, infection, cancer, and hereditary diseases. Bone fractures are considered a major cause of bone injuries and can occur in different parts of the human body, including the skull, spinal, nasal, femur, radius, tibia, and ankle, and are mainly caused by traumatic processes. Bone loss and osteoporosis resulting from ageing are also considered sources of bone damage, and can usually increase the risk of bone fractures as well [17]. Infections such as osteomyelitis are often manifested by pain, redness and fever in specific area, and if not properly treated, may lead to irreversible trauma, such as amputation [18–20]. Additionally, tumors can induce significant bone remodeling, fractures, and anemia, and can even threaten human life under severe circumstances [21]. While surgery is commonly used in bone cancer treatment, it often leads to the generation of defects in the treatment site. Hereditary diseases such as hereditary multiple exostoses (HME) and hereditary bone marrow failure syndromes can also elicit bone injuries, and usually require surgical resection or stem cell transplantation [22,23].

All of the conditions mentioned above can produce bone defects to an extent, but once the defect exceeds the critical size of bone self-healing ability, extraneous intervention is required to promote bone regeneration and to restore normal bone function. Each of these categories of defects needs to be addressed and deeply investigated so as to develop new technologies to effectively treat bone damage.

2.2. Current treatments for bone regeneration

Successfully treating bone fractures requires that the displaced bone be adjusted and fixed to the normal state usually with metallic scaffolds to facilitate correct bone healing. The integration between materials and the natural tissue greatly influences bone regeneration. Materials possessing superiority in biocompatibility and biodegradability can stably anchor damaged bone and promote bone tissue growth, while avoiding an inadvertent immune response. Conventionally, metal implants such as bone nails possess good mechanical strength in treating defects in weight-bearing bones, including femur, tibia and spine [4]. However, metallic scaffolds are bioinert and cannot fully integrate with surrounding tissues. Bioactive and osteoconductive biomaterials such as calcium phosphate (CaP), a main constitute of bone tissues, were later developed to accelerate the bone healing process. For example, metal implants coated with CaP have been found to not only fix the displaced bone, but also allow integration with nearby tissue [24]. Considering its biodegradability, protein-binding affinity and osteoconductivity, a CaP bone graft substitute is thought to be an ideal alternative for bone treatments, and there have been several attempts in CPC grafts [7,25]. However, its biological performance is restricted and can only be applied to non-weight bearing defects, due to limited mechanical strength, poor water-soluble resistance, and uncertain degradation rate and curing time. Hydroxyapatite shows good biocompatibility and can firmly combine with the natural bone. However, its low mechanical strength, poor toughness, and difficulty in controlling pore size and porosity limit its success in bone regeneration.

Table 1 illustrates the types of available materials for bone regeneration, the applications, and merits of each treatment. Although many materials have been explored to replace injured bones and promote bone regeneration, including metal implants, calcium phosphate cements, hydroxyapatite, each one has yet to achieve optimal mechanical and biological performance. Hydrogels, a type of polymer scaffold, have attracted interest in several biomedical fields, including bone repair. We will discuss various promising nontraditional hydrogel matrices that have

Table 1
Commercially available materials for bone regeneration.

Material	Name	Application	Merit	Reference
Autograft	AICBG	Spine fusion	Gold standard	[26,27]
Allograft or demineralized bone matrix (DBM)	Grafton, DBX®, AlloMatrix	Spine fusion, craniofacial bone injury	Osteoinductive, osteoconductive, osteoinductive	[27–29]
BMP and rhBMP	BMP-2, BMP-7; rhBMP-2, OP-1	Open tibial fractures and tibial nonunions	Osteoinduction	[26,28]
Metallic implant	Actipore™	Intervertebral fusion	Biocompatible, porous, shape-memory, super-elastic	[30]
Ceramic	Norian® SRS® Calceon® 6 Osteoset®	Cranialfacial bone defect	Osteoconductive, osteointegrative, biodegradable, nonimmunogenic, porous	[26,31,32]
Bioactive glass	BonAlive	Osteomyelitis	Anti-infective carrier	[33]
Glass-ceramic	Biosilicate®	Femoral condyle bone defect	Osteogenic	[34]
Natural polymers	Healos®	Spinal fusion	Flexible, biocompatible and biodegradable;	[35,36]
Synthetic polymers	Cortoss® OPLA® BoneTec®	Spine fusion, loading-bearing sites	Mechanical strength, controlled degradation	[35,37,38]
Composites	Collagraft®, Collapat®, Vitoss®	Femoral or cancellous, bone defects	Biocompatible, tunable physiochemical properties	[35,39,40]

demonstrated excellent bone formation in different animal defect models and elaborate on their advantages, challenges, and scopes in bone regeneration.

3. Hydrogel-based bone regeneration

In order to promote better bone regeneration, hydrogel-based cell delivery and drug delivery have emerged as potential solutions in tissue engineering and regenerative medicine. They can provide natural hydrophilic three-dimensional environment conducive to cell survival and support new bone growth. Additionally, hydrogels can be tailored to obtain the desired geometry for implantation or injection, and the degradation rate and porosity or release profile can be easily controlled by altering the crosslinking method and degree.

3.1. Requirements for hydrogels used for bone regeneration

Ideally, optimized hydrogel formulations for bone regeneration need to meet the following requirements: 1) noncytotoxic and nonimmunogenic to avoid causing imflammatory response; 2) osteoinductive, osteoconductive, osteogenic, as well as osteocompatible for enhanced bone regeneration; 3) mimic the natural ECM to the greatest degree to facilitate cell adhesion, propagation, and ultimately osteogenic differentiation at implant site; 4) degradable by endogenous enzymes or hydrolysis, synchronizing with new bone ingrowth to make enough space for new bone formation; 5) structural stability and mechanical strength that can be used in treating load-bearing defects and prevent denaturation during sterilization; 6) appropriate pore size and interconnected porosity that can be optimized via altering the concentration and variety of polymers and crosslinkers to enhance cell interaction, control the release of encapsulated bioactive factors, and allow the exchange of nutrients, oxygen, and metabolic waste within the hydrogels; and 7) injectable capability with patient compliance to reduce the pain and simplify the administration process [40].

3.2. Classification of hydrogels employed in bone regeneration

In general, hydrogels can be classified by their sources, preparation methods, crosslinking properties, delivery method, degradability, and so on [41]. In this review, we will focus on several natural and synthetic hydrogels systems that have attracted interest in the scientific world.

3.2.1. Natural materials

Hydrogels can be synthesized from natural materials, including natural proteins (fibrin, fibroin, collagen and gelatin) and polysaccharides (chitosan, hyaluronan and alginate). The natural polymers are either components of, or are similar to the natural ECM, have good biocompatibility, low immune response and cytotoxicity, and can promote cell adhesion, proliferation and new tissue regeneration [42]. The structure of natural polymers is similar to the ECM, thereby providing mechanical stability and structural integrity to bone tissues and preventing inflammatory or immune responses. They can be absorbed through metabolic degradation or enzyme-controlled degradation. Lindsey et al. filled collagen gel into the bone defect in the dorsal nasal bone of the rats [43]. After six weeks, there was a thin bone layer on the surface of the defect, while the healing area of the rats without collagen gel filling was less than 7%, indicating that collagen gel has a positive effect on the repair of the nasal cavity defect. Patterson et al. delivered bone morphogenetic protein (BMP)-2 loaded hyaluronic acid (HA) gel to the cranial defect site of rats, and 75–100% of the BMP was released within the first 24 h [44]. HA gel BMP combination promoted higher bone formation in the defected area of rats than the treatment without HA gel. Several desirable mechanical properties of the material are necessary for the implant in the clinical operation; however, hydrogels made of natural polymers are usually associated with poor mechanical strength and only can be applied to non-weight-bearing sites. Physical or chemical methods (functional, crosslinking and/or copolymerization) to improve the internal specific functional groups, hydrogen bonding, and electrostatic interaction of the natural materials, can increase the materials'

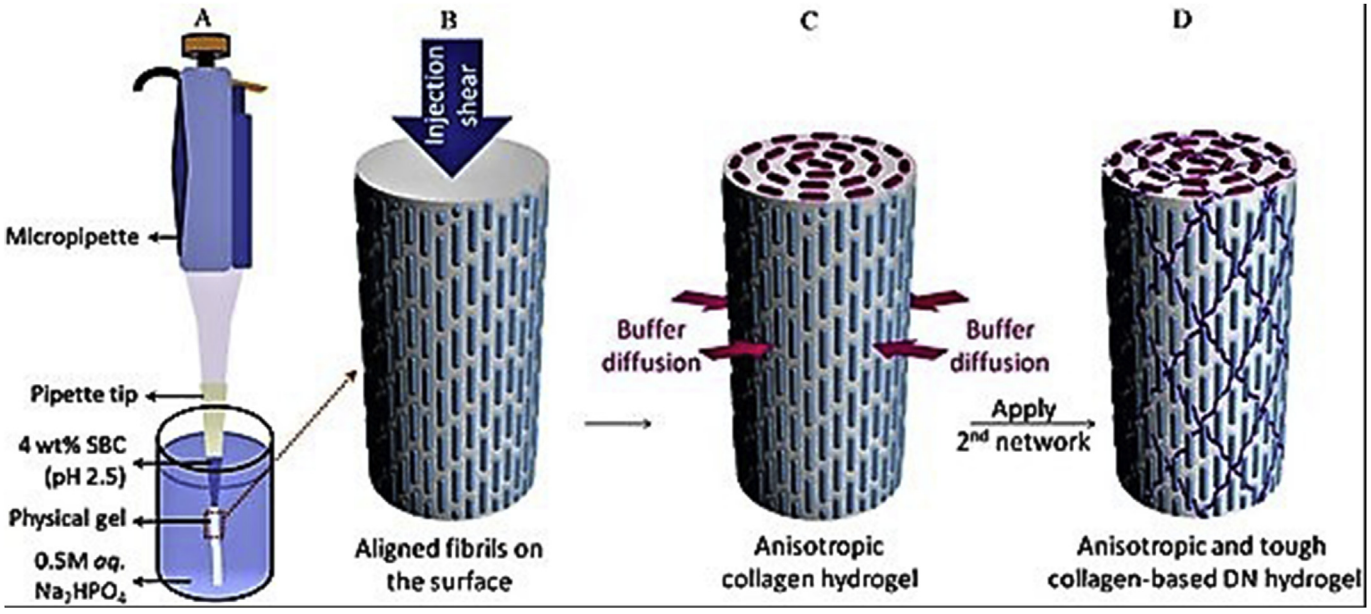

Fig. 2. Method and mechanism to obtain the strong and toughened dual network (DN) hydrogel. (A) The acid SBC was injected into the aqueous solution by using the pipette to form the superstructure. (B) The collagen is quickly injected into the Na_2HPO_4 solution, and the injection shear creates aligned fibrils (blue). (C) Some twisted collagen molecules produce concentrically oriented fibrils (pink) by fibrillogenesis induced syneresis process. (D) The anisotropic SBC gel was immersed in N, N′- two methacrylamide (DMAAm) solution, and DMAAm was polymerized to obtain collagen based anisotropic DN hydrogel (SBC/PDMAAm). Copyright, Ref. [46], 2017, Elsevier.

bioactivity, strength and toughness, thereby expanding the scope of their clinical use [45]. Mredha et al. successfully developed a strong and toughened dual network (DN) hydrogel, in which physically/chemically crosslinked anisotropic swimming bladder collagen (SBC) fibril is the first network, and neutral, biocompatible poly (N, N′- two methacrylamide) (PDMAAm) is the second network [46] (Fig. 2). *In vivo* experiments show that the new DN hydrogel improved the stability of the gel and the strength of the binding to the bone. Kim et al. designed a bionic system for local delivery of drugs made from hyaluronic acid (HA) and vinyl phosphonic acid (VPAc) cross-linked biomineralized hydrogels [47]. By regulating the crosslinking density, mineralization degree, and ionic strength, the system could control the water content, degradation rate, speed of drug release, and could successfully deliver the protein drugs that would promote bone repair and regeneration.

3.2.2. Synthetic materials

Hydrogels used in bone repair and regeneration can be made of biodegradable polymer materials, such as polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyacrylamide (PAM), Sanya methyl carbonate, poly (lactic acid) and its copolymers and so on [48]. Unlike natural materials, synthetic polymers have basic structural units, so the properties of polymers (such as porosity, degradation time, and mechanical properties) can be adjusted for specific applications. The synthetic polymers have reliable material sources and long shelf lives, so they can be produced in large quantities without the risk of immunogenicity [49]. Hydrogels made from synthetic polymers are promising carriers for delivering active proteins, growth factors and drugs to bone tissue. Lee et al. used new hydrogels composed of poly(aldehyde guluronate) (PAG) and adipic acid dihydrazide instead of alginate hydrogels as cell carriers to implant primary rat cranial osteoblasts into the back bone defect in mice [50]. Nine weeks later, mineralized bone tissue formed at the defect. Synthetic polymers have extensive mechanical stiffness and controllable degradation rate. It is reported that the pendant cyclic ester modification of PCL can modulate the slow drug release. The degradation of amphiphilic PCL-PEG-PCL hydrogel resulted from the strong hydrophobicity and crystallinity of PCL segments [51]. The composition of synthetic copolymers has an effect on the structure and properties of the gels. In the preparation of poly (vinylphosphonic acid-co-acrylic acid) (PVPA-co-AA) used as a bone graft substitute, Dey et al. found that increasing PVPA content generated hydrogels with great swelling capacities, high porosities, and adjustable mechanical and cell adhesion properties [52] (Fig. 3). Although synthetic materials have the above advantages, their success is limited by their own inherently poor biological activity, acid by-products, and other shortcomings. Therefore, synthetic materials can be conjugated with biological and chemical entities to improve the comprehensive properties of hydrogels [53]. Thoma et al. divided PEG hydrogels into six groups, according to the density of the gel (physical modification) and the effect of polyethylene glycol (PEG) hydrogels modified with the sequence of RGD (chemical modification). Each group was implanted onto six loci of rabbit skull. After six weeks of observation, they found that chemical and/or physical modification had a significant effect on PEG hydrogel matrix stability, degradation time, and integration into the surrounding soft tissues and hard tissue [54].

4. Various hydrogel structure used in bone regeneration

Hydrogel-based bone repair is contingent on a designing a viable hydrogel formulation that will encapsulate and deliver proteins and bioactive substances. A wide variety of hydrogel configurations can be synthesized via different fabrication techniques. In order to expand the application of hydrogels in the field of bone regeneration, continuous improvement of preparation methods to develop suitable hydrogel formulations for repairing bone defects is imperative. Understanding the complex process of hydrogel synthesis and modifying the matrix to enhance the biocompatibility and osteoconductive, osteoinductive, and osteogenic capabilities of the hydrogel will accelerate the creation of successful configurations. We will elaborate on the design, development, advantages, and limitations of three hydrogel structures: microbeads, nanogels, and hydrogel fibers.

4.1. Hydrogel microbeads

Polymer hydrogels are used for bone regeneration in the form of microbeads, which can be prepared via microfluidics, emulsification, electrostatic droplet extrusion, coaxial air jetting and in-situ polymerization. However, traditional methods do not yield uniform small-sized microbeads. In recent years, researchers have developed a non-equilibrium microfluidic technique for the preparation of smaller-size hydrogel beads (size less than 100 μm). Polymer materials are injected into nonequilibrium W/O interface containing hydrogel molecules, in which the water molecules are dissolved into a continuous phase, and hydrogel precursors in water-in-oil droplets are shrunk and condensed rapidly, forming microbeads smaller than the those formed under the conventional method [55] (Fig. 4). In addition, by adjusting the degree of droplet contraction and the concentration of crosslinking agent, the shape of hydrogel microbeads can be controlled to form non-spherical microbeads. The smaller-sized microbeads have more advantages in achieving high-resolution cell assembly than ordinary sized microbeads.

Due to the significant increase in surface volume ratio compared to the conventional hydrogel, the smaller-sized hydrogel microbeads possess enhanced mass transfer capacity, which is beneficial for the delivery of drugs and stem cells to bone defect sites [56]. Moshaverinia et al. developed an injectable alginate hydrogel microbead to encapsulate dental-derived MSCs, including gingival mesenchymal stem cells (GMSCs) and periodontal ligament stem cells (PDLSCs) [57]. The alginate and stem cell mixture was squeezed into a solution of calcium chloride with syringe for ionic crosslinking. Micro-CT analysis showed that the cells remained viable post-implantation and ectopic mineralization was observed both inside and around the microbeads due to efficient nutrient and oxygen transport. However since the *in vivo* non-enzymatic degradation of alginate microbeads is very lengthy, Leslie and co-workers [58] envisaged incorporating alginate lyase into microbeads to regulate the degradation of alginate. The microbeads were produced using high electrostatic potential without altering the osteogenic differentiation potential of the encapsulated rat adipose derived stem cells (ASCs). Higher levels of osteocalcin expression were observed when compared to microbeads that did not incorporate ASCs or were not exposed to alginate lyase.

Since alginate lacks cell adhesion and osteogenic differentiation, Wang et al. used chitosan and collagen to develop adult bone marrow stem cells loaded and size-adjustable hydrogel microbeads via double crosslinking mechanisms, which exhibited elevated expression of transcription factor osterix and osteocalcin and significant deposition of bone minerals within the osteogenic medium [59]. Another interesting investigation accomplished by Wise and his colleagues utilized water-in-oil emulsion method to develop collagen-chitosan hydrogel microbeads to encapsulate MSCs and BMMCs, which demonstrated a synergistic effect in promoting mineral deposition and significantly enhancing ectopic bone regeneration [60]. The results showed that microbeads containing

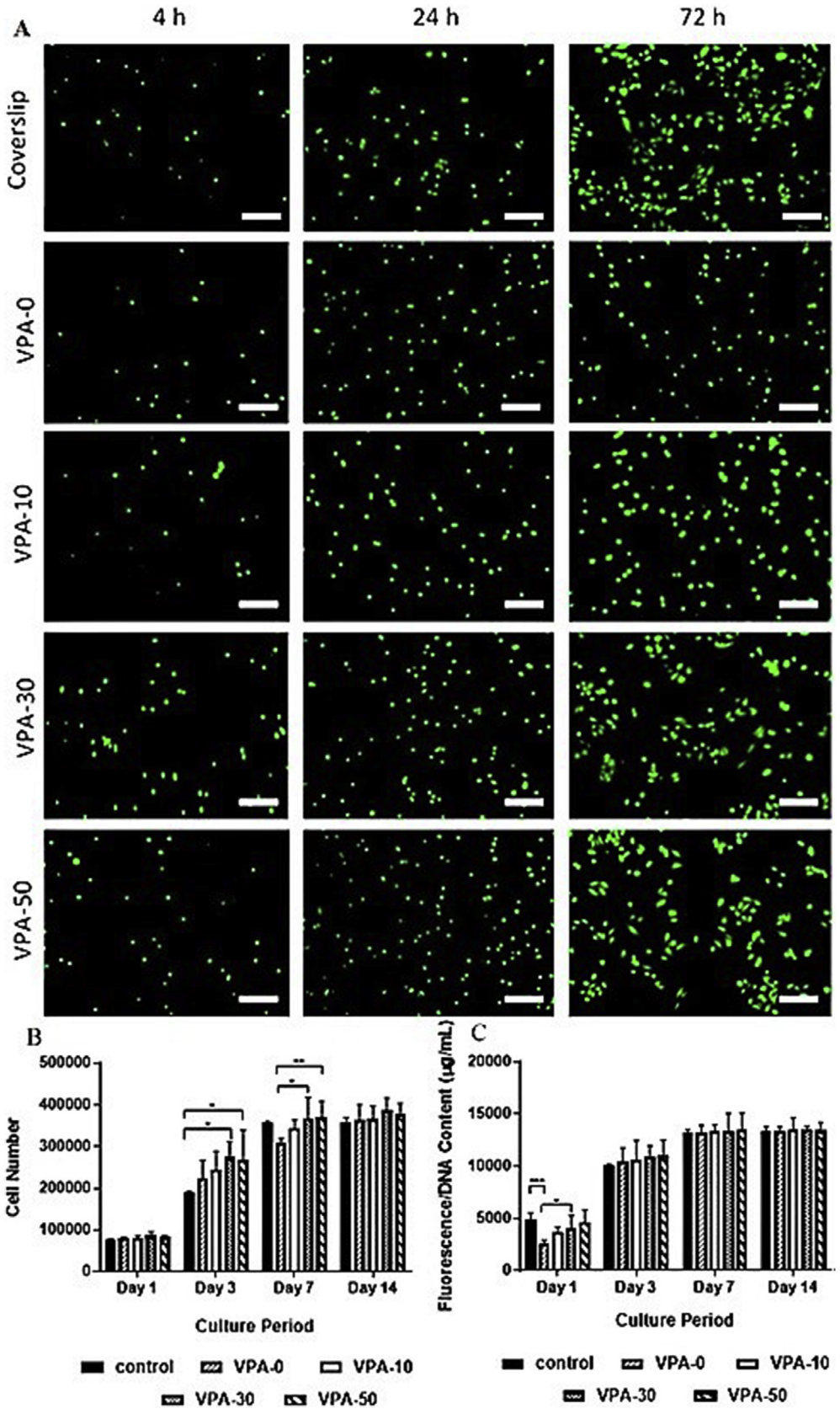

Fig. 3. The effect of poly (vinylphosphonic acid-co-acrylic acid) hydrogels on osteoblast adhesion and proliferation. (A) The Live/Dead human osteoblasts on PVPA-co-AA hydrogels. Live cells stained green, dead cells stained red. (B) The effect of VPA content in PVPA-co-AA hydrogel on the proliferation and metabolic activity of osteoblasts, over 14 days. Copyright, Ref. [52], 2017, Society for Biomaterials.

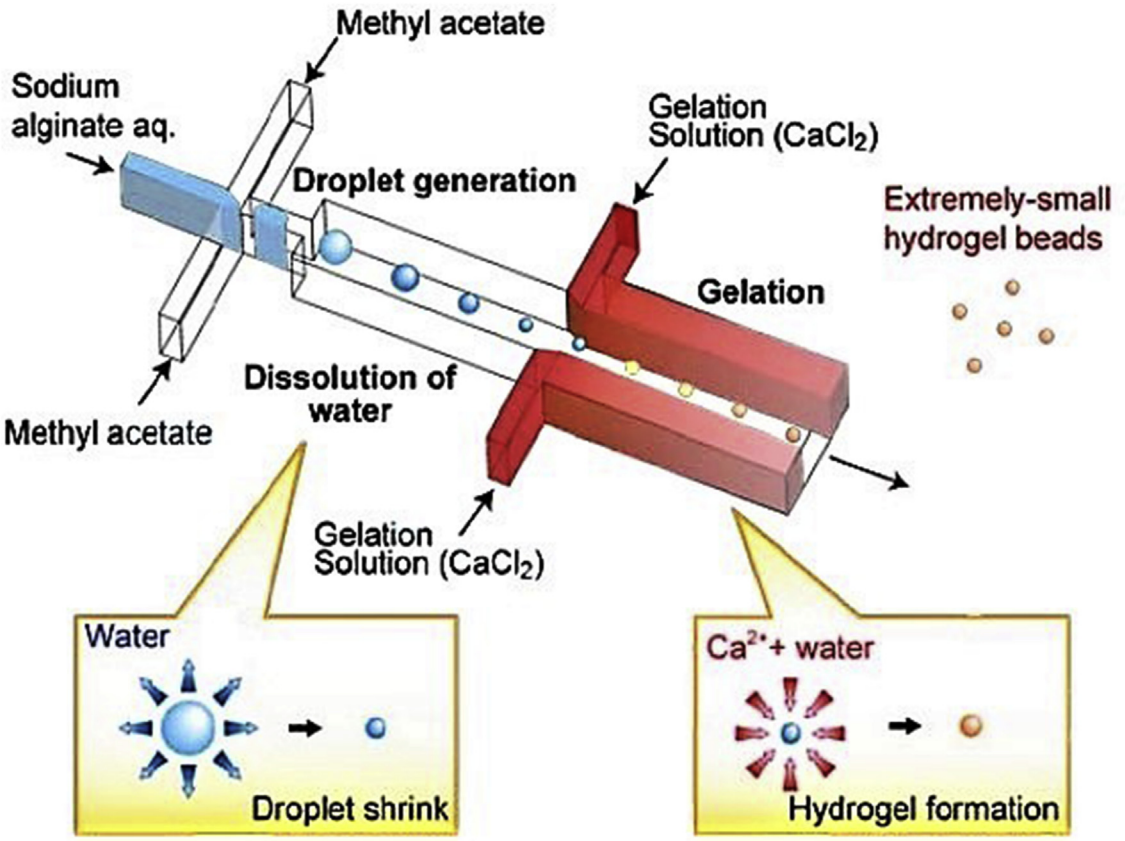

Fig. 4. Schematic diagram of the synthesis of small size hydrogel beads by non-equilibrium microfluidic technology. Ethyl acetate absorbs water of droplets containing sodium alginate and the droplet volumes are decreased. The shrunken droplets by gelation produce extremely-small hydrogel beads. Copyright, Ref. [55], 2011, IEEE.

both MSCs and BMMCs in osteogenic medium (group F + C_O) had remarkable bone volume; however, the tissue mineral density (TMD) was not significantly distinguished, except for the group containing only cultured MSCs and placed in the growth medium (group C_G), in which case the TMD of which was almost zero, as shown in Fig. 5. Microbeads have demonstrated great potential in encapsulating live stem cells and drugs due to their cross-linking mechanisms; however, further investigation must be conducted to develop biocompatible, osteoconductive, osteoinductive, and osteogenic hydrogel microbead formulations.

4.2. Hydrogel nanoparticles

Hydrogel nanoparticles (nanogels) are a group of spherical nanoparticles formed by physically or chemically cross-linking polymer three-dimensional networks that can swell in water. Nanogels are usually synthesized through an emulsion polymerization, such as inverse emulsion polymerization method and distillation-precipitation polymerization, which copolymerizes a rapidly stirred solution at high temperature to obtain stable dispersions [61]. Nanogels have a series of hydrogel characteristics, such as good biocompatibility and mechanical properties, and have a great advantage in the application of bone regeneration. In terms of surface, size, and other aspects, nanogels have the properties of nanoparticles. They are promising responsive carriers for drug delivery due to their uniformity, tunable size, ease in designing and preparation, large surface area of multivalent biological conjugate, high drug loading capacity, and good encapsulation stability. Under mild preparation conditions, nanogels can also deliver proteins without denaturation through the original encapsulation method [62]. A previous study has reported that hydrogel nanoparticles composed of hydrophobized cholesterol-bearing pullulan (CHP) can be used to deliver a variety of hydrophobic proteins and enzymes [63]. Acrylate group–modified CHP nanogels delivered recombinant human fibroblast growth factor 18 (FGF-18) and recombinant human BMP-2 to bone defects and effectively activated bone cells to regenerate bone through combination therapy [64] (Fig. 6). This shows that the nanogels can successfully deliver two different proteins and effectively induce bone repair. Miyahara et al., respectively, used collagen membrane, cholesterol-bearing pullulan (CHP) nanogel membrane and an untreated membrane in healing parietal bone defect of adult Wistar rats. Four weeks later, they observed that the new bone formation of the rat in nanogel group was significantly higher than the other two groups. More interestingly, the newly formed bone in the nanogel group and the original bone could not be histologically distinguished [65]. However, due to the difficulty in controlling the crosslinking point during gel formation, the nanostructure will be non-homogeneous, and the drugs in the nanogels will be released rapidly [66]. Therefore, it is necessary to design a nanoscale structure (bond type, mesh size, crosslinking density, etc.) that will effectively control drug release. Young and his colleagues used the PEG nanogels prepared by inverse microemulsion polymerization (IPMP) as the carrier of a pro-angiogenic peptide, QK. The cross-linking density of the nanogels was controlled by changing the mole fraction of the crosslinker to adjust the release kinetics of QK peptide [67]. Seo et al. developed compact nanogels with diameters less than 200 nm, gelling immediately after in situ injection [68] (Fig. 7). Nanogels composed of hydrophobic isoleucine ethyl ester and hydrophilic polyethylene glycol were found to control the

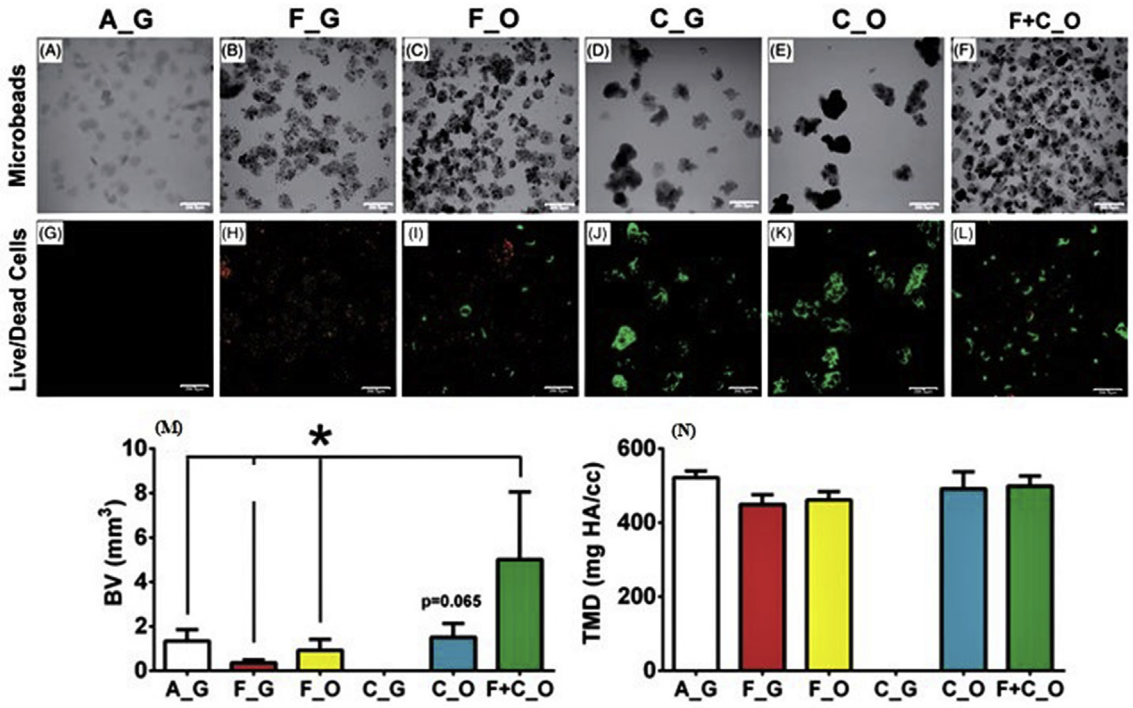

Fig. 5. Cell-encapsulating microbead implants cultured in osteogenic media (O) or growth media (G). (A–F) Morphology of microbead implants cultured in vitro at day 17, imaged by transmitted light microscopy. (G–L) Viability evaluation of microbead implants cultured in vitro at day 17 via cell staining. (M) Bone volume of microbead implants at 5 weeks. (N) Tissue mineral density (TMD) of microbead implants at 5 weeks. A = acellular; F = freshly isolated BMMC; C = culture-expanded MSC. Scale bar = 200 μm. Copyright, Ref. [60], 2016, Taylor & Francis.

release of bone BMP-2 through a hydrophobic interaction and an ionic interaction between BMP-2 and carrier materials. While nanogels have demonstrated success in delivering proteins and growth factors and present great potential in effectively inducing bone growth, designing tunable hydrogels that will provide strong mechanical stability and sustained release is imperative in developing efficacious treatments for bone repair.

4.3. *Hydrogel fibers*

Hydrogel fibers consist of a fibrous structure and a diameter ranging from several nanometers to several microns [69]. The fabrication of a hydrogel fiber usually involves two steps: spinning and crosslinking process. Generally spinning can be divided into various types, including electrospinning [70], wet spinning [71], microfluidic spinning [72], gel spinning [73], 3D printing technology [74] and hydrodynamic spinning [75], among which electrospinning and microfluidic spinning are currently most widely investigated. The operating principle of these two fabrication processes is clearly illustrated in Fig. 8. When fibers are fabricated, they need further crosslinking in the presence of thermal or UV exposure, glutaraldehyde, or enzymes to finally form hydrogel fibers [72].

Compared to microbeads, hydrogel fibers can be aligned axially with the syringe, and can be injected into the defect site; additionally, they can remain at the implant site for a longer period of time [76]. Hydrogel fibers have shown prospective application in tissue engineering, due to their high surface to volume ratio, fast response, and immobilization ability [77]. For example, Perez and his colleagues co-delivered Co and BMP with well-tunable core-shell hydrogel fiber scaffolds and successfully induced osteogenesis and angiogenesis for synergistic bone regeneration in rat calvarium defect, as is shown in Fig. 9 [78]. Gelatin is the partially degraded product of collagen, and due to its naturally binding arginine-glycine-aspartic acid (RGD) peptide, it is considered an excellent polymer for preparing hydrogel fibers with sites for cell attachment [75]. Hu et al. prepared cell-seeded solid or hollow hydrogel fibers by continuously crosslinking a hydrogel precursor solution of gelatin and hydroxybenzoic acid flowing in a multi-phase laminar flow within a three-hole extruder using hydrogen peroxide and horseradish peroxidase [79]. The manufacturing process ensured cell viability and cell dispersion in the hydrogel fibers and has shown great potential in various tissue engineering applications, including bone regeneration.

However, hydrogel fibers also have some non-negligible drawbacks, such as high swelling ratio and poor mechanical strength. Several studies have been conducted to address and resolve these problems. PVA hydrogel fibers generally have high swelling, and as a result, burst release of encapsulated drugs is usually inevitable. In order to alleviate decreased therapeutic effect due to sudden release, a research team successfully extended the duration of drug release through surface modification of hydrogel fibers. Im et al. found that the introduction of C-F bonds onto the surface of PVA electrospun fibers via fluorination can significantly reduce the chance of burst release of Procion Blue and can increase the total release time by 6.7 times [80]. In addition, due to the elongated structure of hydrogel fibers, their mechanical properties are usually not favorable. There have been several efforts to improve their mechanical strength by preparing composite materials of hydrogel fibers, like CPC-hydrogel fiber construct. Wang et al. synthesized a cell-laden CPC–hydrogel fiber composite material using wet spinning technique and following mixation with CPC paste [81]. The mechanical properties and strength of this scaffold surpassed that of cancellous bone, with a strength of 8.5 ± 0.8 Mpa, and demonstrates promise in treating a wide range of bone defects in load-bearing bones. Progress in overcoming the inherent

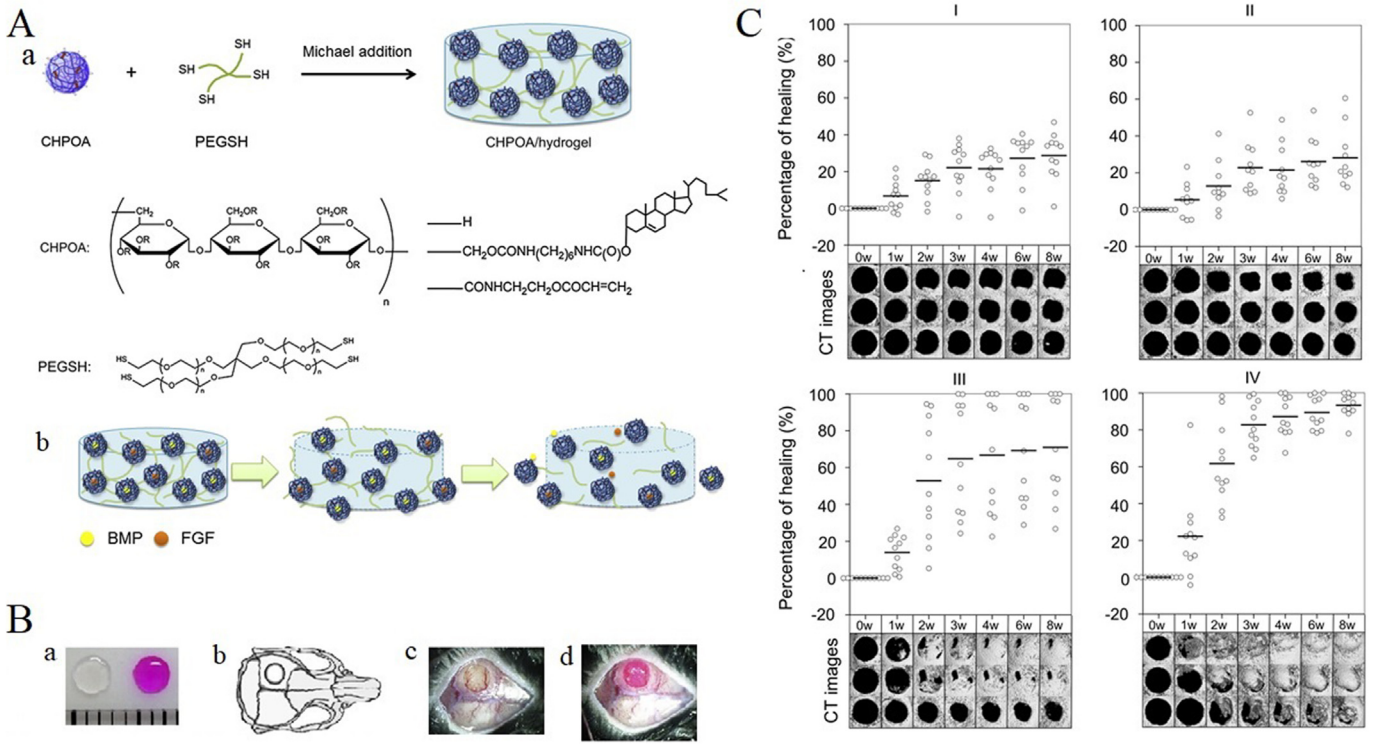

Fig. 6. Cholesteryl group- and acryloyl group-bearing pullulan nanogel to deliver BMP-2 and FGF-18 for bone tissue engineering. (A) Synthesis and decomposition of CHPOA/hydrogel containing FGF-18 and BMP-2. a) The synthesis of CHPOA/hydrogel block and the chemical structure of CHPOA nanogels and PEGSH. b) The process of releasing FGF-18 and BMP-2 by CHPOA nanogels. (B) Disc-shaped CHPOA/hydrogel for mouse skull defect. a) Disc-shaped CHPOA nanogel/hydrogel. Left, Clear standard CHPOA/hydrogel. Right, Pink rhodamine-labeled CHPOA/hydrogel (CHPOA-Rh/hydrogel). b) Schematic drawing of mouse skull from the top. c) Mouse skull formed round bone defects with a biopsy punch. d) the application of CHPOA-Rh/hydrogel to the circular bone defect. (C) The degree of bone healing after CHPOA/hydrogel releasing FGF-18 and/or BMP-2 *in vivo*. Disc-shaped CHPOA/hydrogel pellets: group I, PBS; group II, FGF18 (500 ng); group III, BMP-2 (500 ng); group IV, FGF-18 + BMP-2 (500 ng). The percentage of bone healing in 0, 1, 2, 4, 6 and 8 weeks after implantation of CHPOA/hydrogel. Scatter plots show the data of all the samples. Bar represents the average of each stage. Three typical images of the CT are displayed at the bottom of each scatter plot. Copyright, Ref. [64], 2012, Elsevier.

disadvantages of hydrogel fibers, such as poor mechanical properties and rapid release, is essential to developing a hydrogel formulation that provides controlled prolonged release of delivered proteins and drugs.

5. Hydrogel-based growth factor and stem cell delivery

The delivery of materials for hydrogel-based bone regeneration can be divided according to the different substances encapsulated. It is particularly important that the hydrogel matrix maintains the biological activity of the delivered substances to provide prolonged effective concentrations at the treatment site at a controlled release rate. Growth factors are naturally occurring proteins that are an integral component in promoting bone repair and osteogenesis. A newer technique to induce bone regeneration involves the use of undifferentiated stem cells that will stimulate natural bone formation. We will discuss the promising capabilities and the fundamental challenges associated with two types of delivery systems: growth factor delivery and stem cell delivery.

5.1. Growth factor delivery

Naturally occurring bone healing is a signaling cascade process, involving cells in the defect site secreting cytokines, growth factors, and pro-inflammatory factors and subsequently recruiting surrounding osteogenitor cells to migrate, proliferate, and differentiate into osteoblasts [82]. With a deeper understanding of the mechanism of bone healing, scientists envisage delivering exogenetic bioactive factors into the defect to accelerate bone repair [83]. BMP [84], insulin-like growth factors (IGF) [85], vascular endothelial growth factor (VEGF) [86], stromal cell-derived factor-1α (SDF-1α) [87] and fibroblast growth factor (FGF) [88]are currently the most widely explored in the area of bone regeneration. The variance in structure determines their different physiological functions and specific role in directing new bone growth [82]. Therefore, successful bone repair requires investigators to select appropriate factors in order to obtain desired experimental results.

Since the majority of bioactive factors are proteins, direct delivery may result in enzymatic degradation in the ECM. In addition, the delivered therapeutics may spread to other sites, leading to limited retention at the local site and undesirable adverse effects, such as inflammatory responses or adipogenic induction [89]. Therefore, proper carriers are urgently needed to prevent factors from deactivation. Because hydrogels absorb water, swell, and mimic the natural ECM, they considered as an excellent platform to provide a hydrophilic environment to maintain factors' activity [90]. An interesting synthetic PEG hydrogel functionalized with α2β1 integrin-specific peptide (GFOGER) was developed by Shekaran and co-workers to deliver BMP-2 into murine radial critical-sized defects [91]. The results exhibited that GFOGER hydrogels can protect encapsulated BMP-2, attract osteoprogenitor migration, and promote mechanical bone formation (Fig. 10). Some researchers also explored the impact on bone regeneration through delivering dual factors, as mentioned before, Fujioka-Kobayashi et al. synthesized CHPOA nanogel system containing cholesteryl group and acryloyl group to co-deliver BMP-2 and FGF into mouse calvarial bone defect, leading to the generation of trabecular-like structure [64].

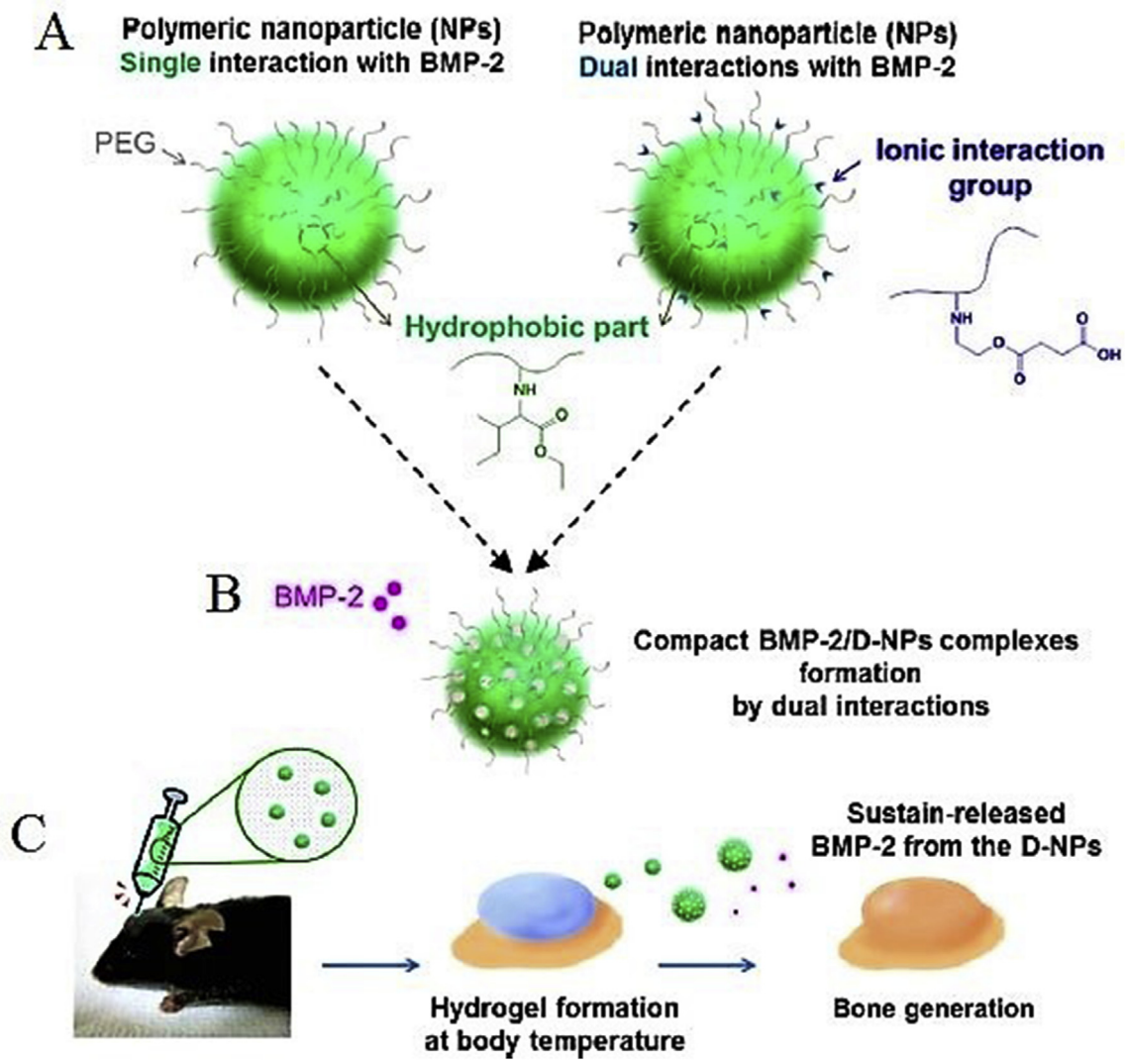

Fig. 7. Sustained BMP-2 delivery of double interacting nanogels for bone regeneration. (A) The D-NPs have hydrophobic group and ionic interaction group. (B) Compact nanocomposites of D-NP and BMP-2 is formed by the interaction of hydrophobic and ionic interactions. (C) After the injection of BMP-2/D-NP nanocomposites, the nanogel is formed and sustainably release BMP-2 locally. A few weeks later, the new bone is generated and the nanogel is completely degraded. Copyright, Ref. [68], 2015, Elsevier.

Ideal hydrogels, due to their unique structure, can also release bioactive factors in a spatiotemporally controlled manner and maintain a sustained release to allow for continuous formation of bone matrix and vascular network [92]. The molecular diffusion and hydrogel degradation rate are the main influencing factors that can be modulated via alteration of the structure and concentrations of polymers and crosslinkers [1]. For example, Seo et al. developed five different thermosensitive poly (phosphazene) hydrogels, all of which have carboxylic acid groups at termini that can combine with BMP-2 through an ionic interaction [93]. However, different length and number of carboxylic acid side chains determined their different physical properties, including sol-gel transition, water absorption, pore size, and BMP-2 release rate. Studies of a critical sized cranial defect model showed that complete defect repair was observed in the BMP-2/PTP-3-L nanocomplex hydrogel group, and the volume and thickness of regenerated bone were 33.0 mm^3 and 0.82 mm, respectively, with BMP-2 fixed at 10 μg. Holloway and his colleagues prepared an HA hydrogel for the delivery of BMP-2 by crosslinking maleimide-modified hyaluronic acid with matrix metalloproteinase-sensitive cell adhesion peptide and later evaluated the influence of hydrogel degradation on *in vivo* new bone formation [94]. The results demonstrated that crosslinking density can affect the compressibility, rheology, and degradability of hydrogel, with lower initial crosslinking density leading to faster BMP-2 release. In addition, hydrogels can be rapidly degraded in the presence of matrix metalloproteinase. Experiments conducted in the rat critical sized calvarial defect model showed obvious new bone formation of BMP-2 loaded hydrogels.

5.2. Stem cell delivery

Since the 20th century, stem cell transplantation has become a research focus in clinical treatment, and has been extensively studied in plastic surgery, vascular diseases and regenerative medicine [95–97]. Stem cells are a group of cells with infinite renewal and differentiation potential, allowing them to differentiate into various cell types, thus laying a solid foundation for their widespread use in different areas [98]. The increased expression of the associated bone-related genes contained in the stem cells further confirmed that the stem cells can differentiate into osteogenic lineage to promote bone repair and regeneration. Stem cells used in bone regeneration mainly contain embryonic stem cells

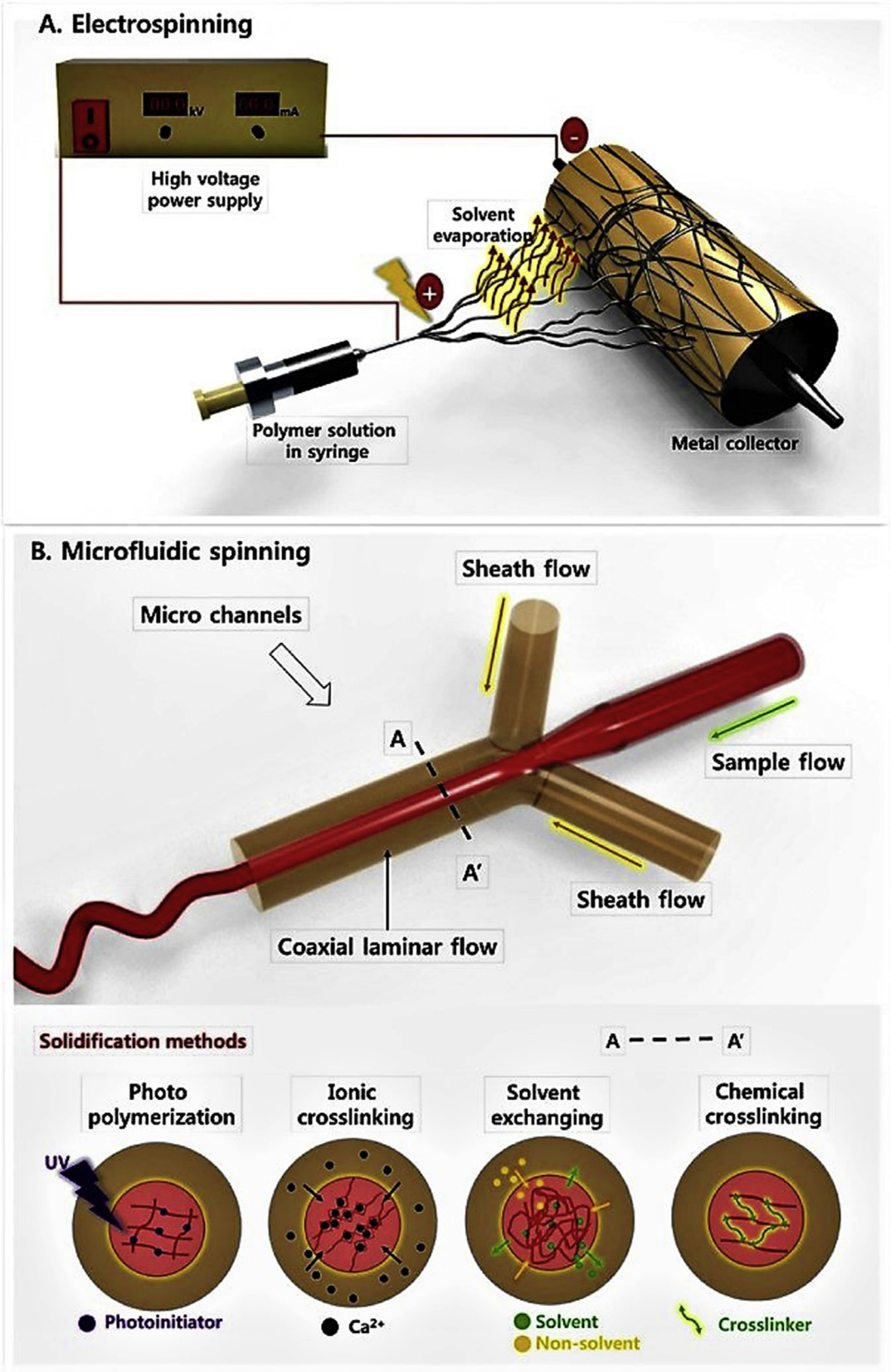

Fig. 8. Operating principle of electrospinning and microfluidic spinning. (A) Electrospinning. (B) Microfluidic spinning. Copyright, Ref. [72], 2017, Elsevier.

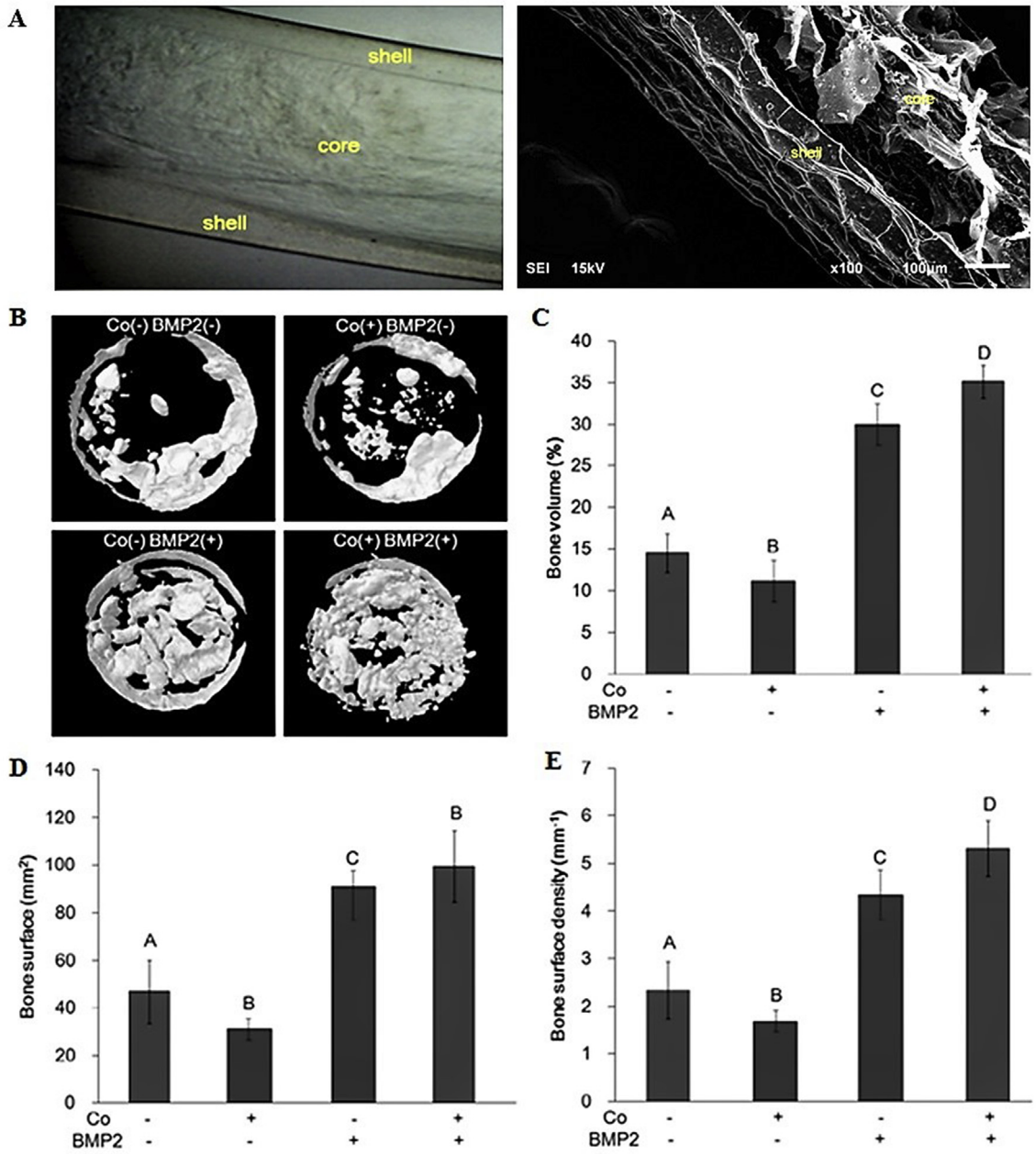

Fig. 9. Core-shell fibrous scaffold for co-deliverling Co and BMP-2. (A) Optical microscopy and SEM images of core-shell hydrogel scaffolds. (B) Microcomputed tomography (μCT) images of new bone formation in rat calvarium defect, treated with fibrous scaffolds. Quantification of (C) new bone volume percentage, (D) bone surface and (E) bone surface density with different combinations of Co and BMP. Copyright, Ref. [78] 2015, Elsevier.

(ESCs) and mesenchymal stem cells (MSCs). Despite successful bone regeneration in previous investigations, ESCs are often inevitably accompanied with controversial moral and ethical issues; later, investigators began to use MSCs to avoid the dilemma [1].

MSCs can not only differentiate into osteoblasts, but also act as a signal center to initiate host response to the bone injury [99]. MSCs can be subdivided into bone marrow mesenchymal cells (BMSCs), ASCs, and umbilical cord mesenchymal stem cells (UCMSCs), all of which can be cultivated and grown in vitro [100]. Among the aforementioned kinds of MSCs, ASCs are most widely investigated because of their easy accessibility, minimal invasion, and low immunogenicity [101]. There have been several investigations concerning directly injecting stem cells into damaged areas. For example, Agung and his colleagues successfully repaired joint damage through intra-articular injection of MSCs, but limited retention and low survival of cells in the injury site due to the sudden change of the environment remains a problem [102]. In order to buffer the irreversible damage to stem cells caused by sudden changes in the external environment after direct injection, researchers began to utilize hydrogels to act as a three-dimensional medium and deliver stem cells [103]. Zhao et al. prepared injectable photocrosslinkable hydrogel microspheres to encapsulate BMSCs by using a microfluidic mixing technique. This approach involved a photoinitiator-containing gelatin-methacryloyl chloride (GelMA), which forms droplets by using a microfluidic device and, followed by UV photocrosslinking, produce GelMA hydrogel microspheres. The BMSCs were then encapsulated into the hydrogels and were found to demonstrate excellent bone formation in rabbit femoral

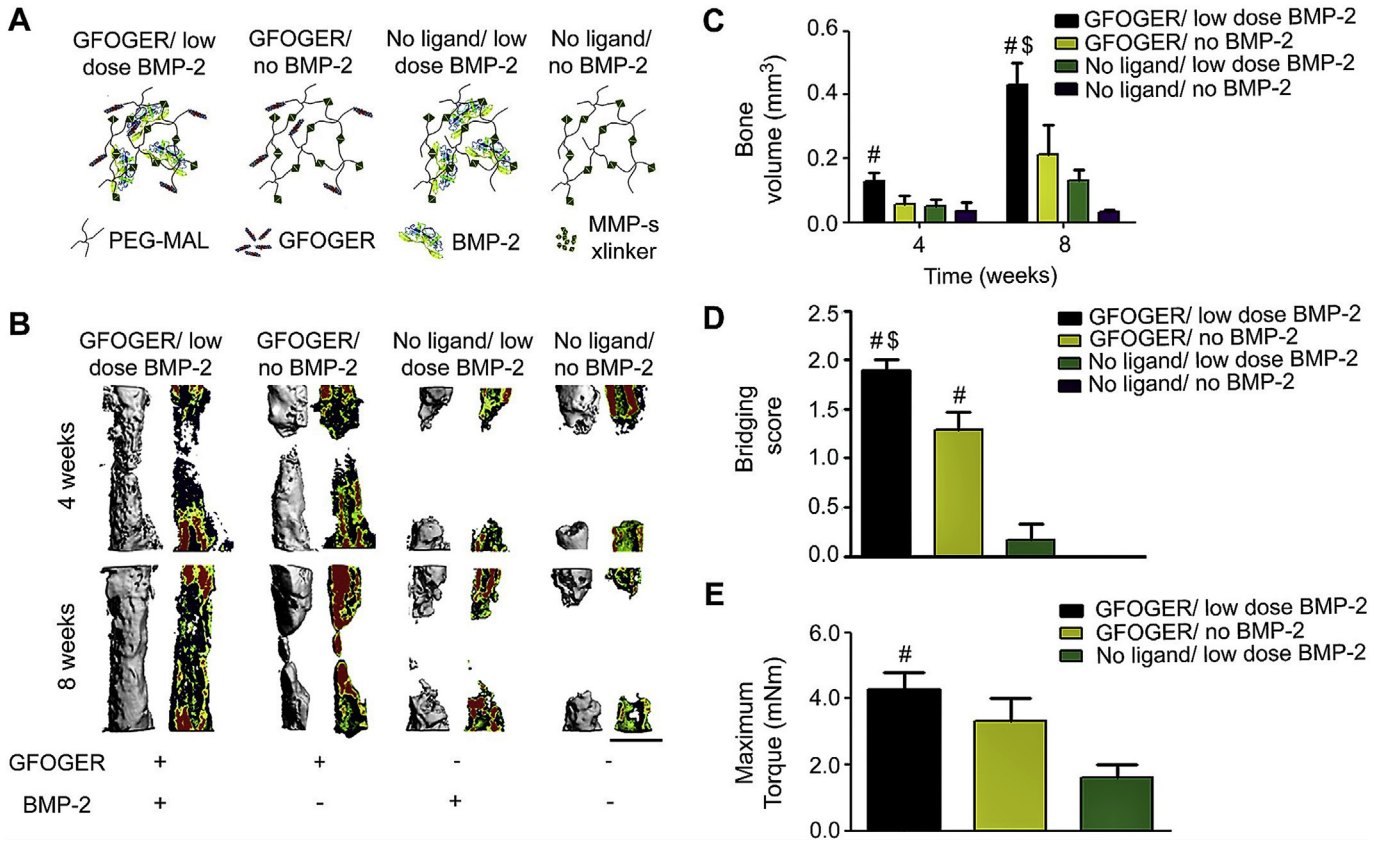

Fig. 10. GFOGER-functionalized PEG synthetic hydrogel encapsulating BMP-2 for treating murine radial defects. (A) Images of hydrogel formulations with different matches of GFOGER and BMP-2. (B) 3D reconstructed images and mineral density mappings of sagittal sections at the same defect when treated with different hydrogel compositions. (C) New bone volume of different hydrogel compositions at 4 and 8 weeks after surgery. (D) Bridging scores of the defects after 4 weeks of surgery. (E) Maximum torque test of different hydrogel formulations at radial defects 8 weeks post-surgery. Copyright, Ref. [91] 2014, Elsevier.

ankle, particularly when BMP-2 was added [104]. In order to circumvent cell death and limited control in the duration of injection and enhance the communication between cells, Huebsch et al. developed interconnected and macroporous hydrogels to improve the transplanted hMSC survival. The animal model used nude rats with cranial defects. The bone regeneration analysis conducted 12 weeks after cell transplantation showed obvious bone formation when hydrogel elasticity was fixed at 60 kPa [105] (Fig. 11)

The interaction between hydrogels and cells is complex, and many factors are involved in the regulation of stem cell differentiation. It has been reported that MSCs prefer to differentiate into osteogenic lineage when stiffness is fixed at 25–40 kPa [106]. Huebsch and his colleagues reported that the matrix elasticity of hydrogels composed of alginate polymers had significant impact on the differentiation of clonally derived hMSCs with intermediate elastic modulus (11–30 kPa) leading to osteogenic differentiation and softer (2.5–5 kPa) inducing adipogenic lineage [107]. Chaudhuri et al. reported that stress relaxation has certain effects on regulating stem cell fate and activity. Faster relaxation of hydrogels can enhance the osteogenic differentiation of the encapsulated MSCs and forms a mineralized matrix which is abundant in collegen-1 and similar to the bone composition [108]. Some researchers proposed that the surface properties of hydrogel will largely affect its interaction with cells, thus regulating the fate of the cells. Benoit et al. formulated a series of materials with different surface charges and hydrophilicities [109]. The results showed that small functional groups added to the hydrogel matrix have a considerable influence on hMSC differentiation with charged phosphate groups inducing osteogenesis and hydrophobic tert-butyl leading to adipogenesis.

6. Challenges and prospects

Successful bone regeneration requires a coordinated interaction among cells, growth factors, and hydrogels. Although hydrogels display inherent advantages in bone regeneration, there are still several problems that must be resolved. Firstly, when designing hydrogels, the biocompatibility should be considered in order to circumvent possible inflammatory response [110]. Natural polymers like collagen, gelatin, and chitosan are widely considered to be biocompatible, but are limited by poor mechanical strength and structural stability and burst release of encapsulated proteins or cells after being delivered to the target site. Synthetic polymers can solve the aforementioned problems to a certain extent, but are accompanied with issues such as unintended immune responses and poor degradation and cell attachment. Therefore, the optimization of polymer composition, concentration and crosslinking methods still needs to be further studied to better promote bone regeneration [111].

Ideally, the encapsulation of natural stem cells and growth factors in the hydrogel matrix will increase the rate of new ECM generation [112]. One of the limitations could be rapid hydrogel degradation prior to the generation of new ECM, thus damaging the mechanical stability and disrupting the process of healing bone defects [113]. Additionally, proper control of stem cell differentiation is crucial to ensure differentiation at the desired cell lineages and prevent undesirable side effects when delivering stem cells to the defect site [114]. Since the ultimate goal of hydrogels is to

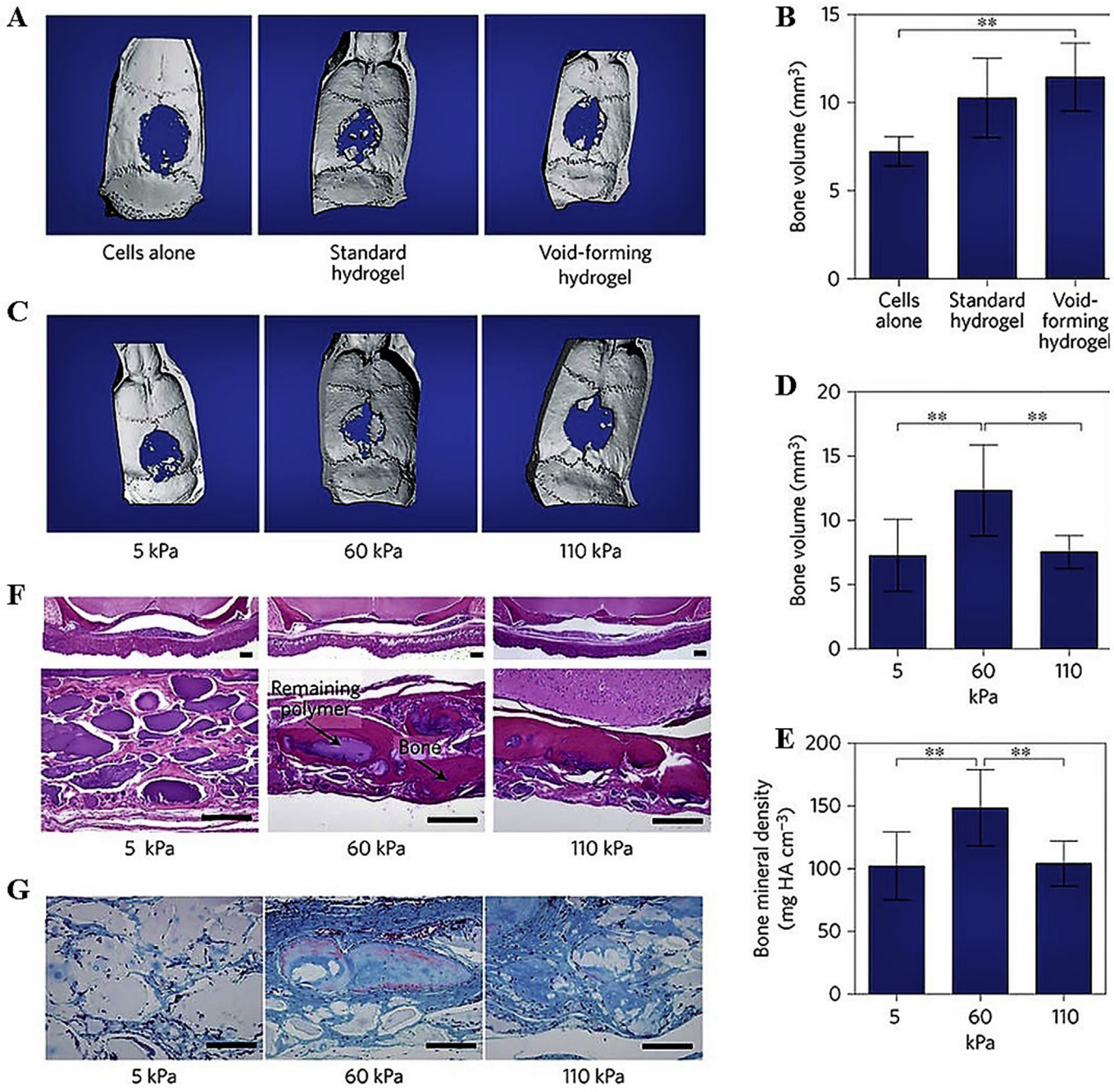

Fig. 11. Matrix elasticity modulates the bone regeneration ability of hMSC-encapsulated hydrogels. (A) micro-computed tomographic (μCT) images of bone regeneration in cranial defects in nude rats 12 weeks post-transplantation of different hydrogels (cells alone, standard hydrogel, void-forming hydrogel). (B) Quantitative analysis of new bone volume by μCT within different hydrogels. (C) μCT images of new bone formation in nude rat cranial defects 12 weeks after transplantation of hMSCs-incorporated void-forming hydrogels fixed at various elastic moduli. Evaluation of new bone volume (D) and bone mineral density (E) in nude rat cranial defects 12 weeks post-transplantation of hydrogels with different elastic moduli. (F) Haematoxylin–eosin (H&E) staining images of newly formed bone and remaining polymers. (G) Masson's trichrome staining results showing bone regeneration. Copyright, Ref. [105] 2015, Nature Publishing Group.

enhance de novo bone formation, the prepared hydrogels should have good interaction with the surrounding tissues – osteointegration – which can be assured by binding integrin ligands onto the surface of hydrogel implants. Several studies have also demonstrated that defect sites lack blood vessels; hence, it is feasible to incorporate angiogenic factors into hydrogels for enhanced bone repair and regeneration [115].

A substantial challenge concerning the uncontrolled release of loaded bioactive growth factors and stem cells, which is directedly related to the hydrogel degradation rate, limits the scope of bone regeneration. Recently, smart hydrogel systems with on-demand delivery capability has become an emerging innovative technology in bone regeneration. Stimuli-responsive hydrogels can sense stimuli in their external environment and make corresponding changes. They have been widely used in biomedicine, tissue engineering, and immobilization of enzymes. The studied external stimuli mainly include temperature, light and pH [116,117]. Development of hydrogels responsive to biochemical stimuli like

enzymes, antigens, and ligands have also been explored [118,119]. Stimuli-responsive hydrogel delivery platform has been shown to demonstrate great potential in repairing bone defects caused by accidents, cancer, or age, and must be investigated and optimized further to control the release of bioactive payload upon changes in the surrounding physiological environment of the body.

Despite the significant existing challenges, including rapid degradation and burst release, poor integration with native cells, low mechanical stability and immunogenicity, the development of hydrogel-based bone regeneration holds enormous promise for the future treatment of bone-related diseases and defects. With a deepening understanding of hydrogels, bone defects, the ECM and their interactions, hydrogels will undoubtedly become a powerful tool for the clinical treatment of bone defects in the future.

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References

[1] T.N. Vo, F.K. Kasper, A.G. Mikos, Strategies for controlled delivery of growth factors and cells for bone regeneration, Adv. Drug Deliv. Rev. 64 (2012) 1292–1309.
[2] M.A. Velasco, C.A. Narvaez-Tovar, D.A. Garzon-Alvarado, Design, materials, and mechanobiology of biodegradable scaffolds for bone tissue engineering, BioMed Res. Internat. 2015 (2015) 729076.
[3] M.S. Simeonov, A.A. Apostolov, E.D. Vassileva, In situ calcium phosphate deposition in hydrogels of poly(acrylic acid)-polyacrylamide interpenetrating polymer networks, RSC Adv. 6 (2016) 16274–16284.
[4] R. Agarwal, A.J. García, Biomaterial strategies for engineering implants for enhanced osseointegration and bone repair, Adv. Drug Deliv. Rev. 94 (2015) 53–62.
[5] A. Forlino, J.C. Marini, Osteogenesis imperfecta, Lancet 387 (2016) 1657–1671.
[6] J.S. Smolen, D. Aletaha, I.B. McInnes, Rheumatoid arthritis, Lancet 388 (2016) 2023–2038.
[7] W. Habraken, P. Habibovic, M. Epple, M. Bohner, Calcium phosphates in biomedical applications: materials for the future? Mater. Today 19 (2016) 69–87.
[8] J. Street, M. Bao, L. deGuzman, S. Bunting, F.V. Peale, N. Ferrara Jr., H. Steinmetz, J. Hoeffel, J.L. Cleland, A. Daugherty, N. van Bruggen, H.P. Redmond, R.A. Carano, E.H. Filvaroff, Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 9656–9661.
[9] K. Lee, C.K. Chan, N. Patil, S.B. Goodman, Cell therapy for bone regeneration–bench to bedside, J. Biomed. Mater. Res. B Appl. Biomater. 89 (2009) 252–263.
[10] D. Puppi, F. Chiellini, A.M. Piras, E. Chiellini, Polymeric materials for bone and cartilage repair, Prog. Polym. Sci. 35 (2010) 403–440.
[11] E. Quinlan, A. Lopez-Noriega, E. Thompson, H.M. Kelly, S.A. Cryan, F.J. O'Brien, Development of collagen-hydroxyapatite scaffolds incorporating PLGA and alginate microparticles for the controlled delivery of rhBMP-2 for bone tissue engineering, J. Contr. Release 198 (2015) 71–79.
[12] S. Wu, X. Liu, K.W.K. Yeung, C. Liu, X. Yang, Biomimetic porous scaffolds for bone tissue engineering, Mater. Sci. Eng. R Rep. 80 (2014) 1–36.
[13] G. Wu, C. Feng, J. Quan, Z. Wang, W. Wei, S. Zang, S. Kang, G. Hui, X. Chen, Q. Wang, In situ controlled release of stromal cell-derived factor-1alpha and antimiR-138 for on-demand cranial bone regeneration, Carbohydr. Polym. 182 (2018) 215–224.
[14] R. Silva, B. Fabry, A.R. Boccaccini, Fibrous protein-based hydrogels for cell encapsulation, Biomaterials 35 (2014) 6727–6738.
[15] T. Gong, J. Xie, J. Liao, T. Zhang, S. Lin, Y. Lin, Nanomaterials and bone regeneration, Bone Res 3 (2015) 15029.
[16] E.M. Bueno, J. Glowacki, Cell-free and cell-based approaches for bone regeneration, Nat. Rev. Rheumatol. 5 (2009) 685–697.
[17] J.L. Carrington, Aging bone and cartilage: cross-cutting issues, Biochem. Biophys. Res. Commun. 328 (2005) 700–708.
[18] B. Baroli, From natural bone grafts to tissue engineering therapeutics: brainstorming on pharmaceutical formulative requirements and challenges, J. Pharmaceut. Sci. 98 (2009) 1317–1375.
[19] D.P. Lew, F.A. Waldvogel, Osteomyelitis, N. Engl. J. Med. 336 (1997) 999–1007.
[20] A. Wijewardena, E. Vandervord, S.S. Lajevardi, J. Vandervord, C.J. Jackson, Combination of activated protein C and topical negative pressure rapidly regenerates granulation tissue over exposed bone to heal recalcitrant orthopedic wounds, Int. J. Low. Extrem. Wounds 10 (2011) 146.
[21] J.M. Jimenez-Andrade, W.G. Mantyh, A.P. Bloom, A.S. Ferng, C.P. Geffre, P.W. Mantyh, Bone cancer pain, Ann. N. Y. Acad. Sci. 1198 (2010) 173–181.
[22] G. Duncan, C. McCormick, F. Tufaro, The link between heparan sulfate and hereditary bone disease: finding a function for the EXT family of putative tumor suppressor proteins, J. Clin. Invest. 108 (2001) 511–516.
[23] R. Bizzetto, C. Bonfim, V. Rocha, G. Socie, F. Locatelli, K. Chan, O. Ramirez, J. Stein, S. Nabhan, E. Miranda, J. Passweg, C.A. de Souza, E. Gluckman, Eurocord, S.-W.f. EBMT, Outcomes after related and unrelated umbilical cord blood transplantation for hereditary bone marrow failure syndromes other than Fanconi anemia, Haematologica 96 (2011) 134–141.
[24] M.M. Saleh, A.H. Touny, M.A. Al-Omair, M.M. Saleh, Biodegradable/biocompatible coated metal implants for orthopedic applications, Bio Med. Mater. Eng. 27 (2016) 87–99.
[25] P. Ducheyne, Q. Qiu, Bioactive ceramics: the effect of surface reactivity on bone formation and bone cell function, Biomaterials 20 (1999) 2287–2303.
[26] A. Nauth, J. Lane, J.T. Watson, P. Giannoudis, Bone graft substitution and augmentation, J. Orthop. Trauma 29 (2015) S34–S38.
[27] T.S. Fu, I.C. Wang, M.L. Lu, M.K. Hsieh, L.H. Chen, W.J. Chen, The fusion rate of demineralized bone matrix compared with autogenous iliac bone graft for long multi-segment posterolateral spinal fusion, BMC Muscoskel. Disord. 17 (2016) 3.
[28] A. Gupta, N. Kukkar, K. Sharif, B.J. Main, C.E. Albers, S.F. El-Amin Iii, Bone graft substitutes for spine fusion: a brief review, World J. Orthoped. 6 (2015) 449–456.
[29] J.A. Leupold, W.R. Barfield, Y.H. An, L.A. Hartsock, A comparison of ProOsteon, DBX, and collagraft in a rabbit model, J. Biomed. Mater. Res. B Appl. Biomater. 79 (2006) 292–297.
[30] A. Bansiddhi, T.D. Sargeant, S.I. Stupp, D.C. Dunand, Porous NiTi for bone implants: a review, Acta Biomater. 4 (2008) 773–782.
[31] A. Oryan, S. Alidadi, A. Moshiri, N. Maffulli, Bone regenerative medicine: classic options, novel strategies, and future directions, J. Orthop. Surg. Res. 9 (2014) 27.
[32] V. Kattimani, K.P. Lingamaneni, P.S. Chakravarthi, T.S. Kumar, A. Siddharthan, Eggshell-derived hydroxyapatite: a new era in bone regeneration, J. Craniofac. Surg. 27 (2016) 112–117.
[33] T.A. van Vugt, J. Geurts, J.J. Arts, Clinical application of antimicrobial bone graft substitute in osteomyelitis treatment: a systematic review of different bone graft substitutes available in clinical treatment of osteomyelitis, Bio-Med Res. Int. 2016 (2016), 6984656.
[34] C.I. van Houdt, C.R. Tim, M.C. Crovace, E.D. Zanotto, O. Peitl, D.J. Ulrich, J.A. Jansen, N.A. Parizotto, A.C. Renno, J.J. van den Beucken, Bone regeneration and gene expression in bone defects under healthy and osteoporotic bone conditions using two commercially available bone graft substitutes, Biomed. Mater. 10 (2015) 035003.
[35] R.Y. Basha, M. Doble, Design of biocomposite materials for bone tissue regeneration, Mater. Sci. Eng. C 57 (2015) 452–463.
[36] C. Laurencin, Y. Khan, S.F. El-Amin, Bone graft substitutes, Expet Rev. Med. Dev. 3 (2006) 49–57.
[37] Z. Dahabreh, M. Panteli, I. Pountos, M. Howard, P. Campbell, P.V. Giannoudis, Ability of bone graft substitutes to support the osteoprogenitor cells: an in-vitro study, World J. Stem Cell. 6 (2014) 497–504.
[38] W. Bojar, T. Ciach, M. Kucharska, J. Maurin, B.M. Gruber, J. Krzyszton-Russjan, I. Bubko, E.L. Anuszewska, Cytotoxicity Evaluation and Crystallochemical Analysis of a Novel and Commercially Available Bone Substitute Material, Adv. Clin. Exp. Med. 24 (2015) 511–516.
[39] M. Havener, L. Brown, M. Darmoc, R. Owsiany, T. Clineff, Improvements in healing with a bioactive bone graft substitute in a canine metaphyseal defect, in: 55th Annual Meeting of the Orthopedic Research Society, Las Vegas, NV, 2009.
[40] S.H. Lee, H. Shin, Matrices and scaffolds for delivery of bioactive molecules in bone and cartilage tissue engineering, Adv. Drug Deliv. Rev. 59 (2007) 339–359.
[41] F. Ullah, M.B. Othman, F. Javed, Z. Ahmad, H. Md Akil, Classification, processing and application of hydrogels: a review, Mat. sci. eng. C, Mater. Sci. Eng. C, Mat. biol. appl. 57 (2015) 414–433.
[42] W. Zhao, X. Jin, Y. Cong, Y.Y. Liu, J. Fu, Degradable natural polymer hydrogels for articular cartilage tissue engineering, J. Chem. Technol. Biotechnol. 88 (2013) 327–339.
[43] W.H. Lindsey, R.C. Ogle, R.F. Morgan, R.W. Cantrell, T.M. Sweeney, Nasal reconstruction using an osteoconductive collagen gel matrix, Arch. Otolaryngol. Head Neck Surg. 122 (1996) 37–40.
[44] J. Patterson, R. Siew, S.W. Herring, A.S.P. Lin, R. Guldberg, P.S. Stayton, Hyaluronic acid hydrogels with controlled degradation properties for oriented bone regeneration, Biomaterials 31 (2010) 6772–6781.
[45] V. Guarino, T. Caputo, R. Altobelli, L. Ambrosio, Degradation properties and metabolic activity of alginate and chitosan polyelectrolytes for drug delivery and tissue engineering applications, Aims Materials Science 2 (2015) 497–502.
[46] M.T.I. Mredha, N. Kitamura, T. Nonoyama, S. Wada, K. Goto, X. Zhang, T. Nakajima, T. Kurokawa, Y. Takagi, K. Yasuda, J.P. Gong, Anisotropic tough

double network hydrogel from fish collagen and its spontaneous in vivo bonding to bone, Biomaterials 132 (2017) 85–95.
[47] S.Y. Kim, J.S. Park, Biomineralized hyaluronic acid/poly(vinylphosphonic acid) hydrogel for bone tissue regeneration, J. Appl. Polym. Sci. 131 (2014) 11.
[48] M.I. Sabir, X. Xu, L. Li, A review on biodegradable polymeric materials for bone tissue engineering applications, J. Mater. Sci. 44 (2009) 5713–5724.
[49] B. Dhandayuthapani, Y. Yoshida, T. Maekawa, D.S. Kumar, Polymeric scaffolds in tissue engineering application: a review, International Journal of Polymer Science 2011 (2011) 1–19.
[50] K.Y. Lee, E. Alsberg, D.J. Mooney, Degradable and injectable poly(aldehyde guluronate) hydrogels for bone tissue engineering, J. Biomed. Mater. Res. 56 (2001) 228–233.
[51] W. Wang, L. Deng, S. Liu, X. Li, X. Zhao, R. Hu, J. Zhang, H. Han, A. Dong, Adjustable degradation and drug release of a thermosensitive hydrogel based on a pendant cyclic ether modified poly(epsilon-caprolactone) and poly(ethylene glycol)co-polymer, Acta Biomater. 8 (2012) 3963–3973.
[52] R.E. Dey, I. Wimpenny, J.E. Gough, D.C. Watts, P.M. Budd, Poly (vinylphosphonic acid-co-acrylic acid) hydrogels: the effect of copolymer composition on osteoblast adhesion and proliferation, J. Biomed. Mater. Res. 106 (2018) 255–264.
[53] E. Dawson, G. Mapili, K. Erickson, S. Taqvi, K. Roy, Biomaterials for stem cell differentiation, Adv. Drug Deliv. Rev. 60 (2008) 215–228.
[54] D.S. Thoma, F.E. Weber, S.P. Bienz, Y. Ge, C.H. Hämmerle, R.E. Jung, Biodegradation and tissue integration of various polyethylene glycol matrices: a comparative study in rabbits, Clin. Oral Implants Res. 28 (11) (2017) e244–e251.
[55] S. Sugaya, A. Miyama, M. Yamada, M. Seki, Fabrication of Functional Hydrogel Microbeads Utilizing Non-equilibrium Microfluidics for Biological Applications, in: 2011 International Symposium on Micro-NanoMechatronics and Human Science, IEEE, 2011, pp. 75–78 .
[56] S. Sugaya, M. Yamada, M. Seki, Manipulation of cells and cell spheroids using collagen hydrogel microbeads prepared by microfluidic devices, in: 2012 International Symposium on Micro-NanoMechatronics and Human Science, IEEE, 2012, pp. 435–438.
[57] A. Moshaverinia, C. Chen, K. Akiyama, X. Xu, W.W.L. Chee, S.R. Schricker, S. Shi, Encapsulated dental-derived mesenchymal stem cells in an injectable and biodegradable scaffold for applications in bone tissue engineering, J. Biomed. Mater. Res. 101 (2013) 3285–3294.
[58] S.K. Leslie, D.J. Cohen, J. Sedlaczek, E.J. Pinsker, B.D. Boyan, Z. Schwartz, Controlled release of rat adipose-derived stem cells from alginate microbeads, Biomaterials 34 (2013) 8172–8184.
[59] L. Wang, R.R. Rao, J.P. Stegemann, Delivery of mesenchymal stem cells in chitosan/collagen microbeads for orthopedic tissue repair, Cells Tissues Organs 197 (2013) 333–343.
[60] J.K. Wise, A.I. Alford, S.A. Goldstein, J.P. Stegemann, Synergistic enhancement of ectopic bone formation by supplementation of freshly isolated marrow cells with purified MSC in collagen-chitosan hydrogel microbeads, Connect. Tissue Res. 57 (2016) 516–525.
[61] S.V. Vinogradov, T.K. Bronich, A.V. Kabanov, Nanosized cationic hydrogels for drug delivery: preparation, properties and interactions with cells, Adv. Drug Deliv. Rev. 54 (2002) 135–147.
[62] H. Zhang, Y.J. Zhai, J. Wang, G.X. Zhai, New progress and prospects: the application of nanogel in drug delivery, Mat. S. Eng. C-Mat. Biol. Appl. 60 (2016) 560–568.
[63] G.S. Themistocleous, H.A. Katopodis, L. Khaldi, A. Papalois, C. Doillon, A. Sourla, P.N. Soucacos, M. Koutsilieris, Implants of type I collagen gel containing MG-63 osteoblast-like cells can act as stable scaffolds stimulating the bone healing process at the sites of the surgically-produced segmental diaphyseal defects in male rabbits, In Vivo 21 (2007) 69–76.
[64] M. Fujioka-Kobayashi, M.S. Ota, A. Shimoda, K.-i. Nakahama, K. Akiyoshi, Y. Miyamoto, S. Iseki, Cholesteryl group- and acryloyl group-bearing pullulan nanogel to deliver BMP2 and FGF18 for bone tissue engineering, Biomaterials 33 (2012) 7613–7620.
[65] T. Miyahara, M. Nyan, A. Shimoda, Y. Yamamoto, S. Kuroda, M. Shiota, K. Akiyoshi, S. Kasugai, Exploitation of a novel polysaccharide nanogel cross-linking membrane for guided bone regeneration (GBR), Regen. Med. Tissue Eng. 6 (2012) 666–672.
[66] Y. Sasaki, K. Akiyoshi, Self-assembled nanogel engineering for advanced biomedical technology, Chem. Lett. 41 (2012) 202–208.
[67] D.A. Young, M.B. Pimentel, L.D. Lima, A.F. Custodio, W.C. Lo, S.C. Chen, F. Teymour, G. Papavasiliou, Design and characterization of hydrogel nanoparticles with tunable network characteristics for sustained release of a VEGF-mimetic peptide, Biomater. Sci. 5 (2017) 2079–2092.
[68] B.B. Seo, H. Choi, J.T. Koh, S.C. Song, Sustained BMP-2 delivery and injectable bone regeneration using thermosensitive polymeric nanoparticle hydrogel bearing dual interactions with BMP-2, J. Contr. Release 209 (2015) 67–76.
[69] M. Ignatova, N. Manolova, N. Markova, I. Rashkov, Electrospun non-woven nanofibrous hybrid mats based on chitosan and PLA for wound-dressing applications, Macromol. Biosci. 9 (2009) 102–111.
[70] M. Guvendiren, J.A. Burdick, Engineering synthetic hydrogel microenvironments to instruct stem cells, Curr. Opin. Biotechnol. 24 (2013) 841–846.
[71] S. Sugaya, M. Yamada, A. Hori, M. Seki, Microfluidic production of single micrometer-sized hydrogel beads utilizing droplet dissolution in a polar solvent, Biomicrofluidics 7 (2013), 054120.
[72] J. Cheng, Y. Jun, J. Qin, S.H. Lee, Electrospinning versus microfluidic spinning of functional fibers for biomedical applications, Biomaterials 114 (2017) 121–143.
[73] M. Tang, W. Chen, M.D. Weir, W. Thein-Han, H.H.K. Xu, Human embryonic stem cell encapsulation in alginate microbeads in macroporous calcium phosphate cement for bone tissue engineering, Acta Biomater. 8 (2012) 3436–3445.
[74] H.Y. Lin, C.W. Peng, W.W. Wu, Fibrous hydrogel scaffolds with cells embedded in the fibers as a potential tissue scaffold for skin repair, J. Mater. Sci. Mater. Med. 25 (2014) 259–269.
[75] M. Hu, R. Deng, K.M. Schumacher, M. Kurisawa, H. Ye, K. Purnamawati, J.Y. Ying, Hydrodynamic spinning of hydrogel fibers, Biomaterials 31 (2010) 863–869.
[76] Y.J. Heo, H. Shibata, T. Okitsu, T. Kawanishi, S. Takeuchi, Long-term in vivo glucose monitoring using fluorescent hydrogel fibers, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 13399–13403.
[77] P. Lu, Y.-L. Hsieh, Organic compatible polyacrylamide hydrogel fibers, Polymer 50 (2009) 3670–3679.
[78] R.A. Perez, J.H. Kim, J.O. Buitrago, I.B. Wall, H.W. Kim, Novel therapeutic core-shell hydrogel scaffolds with sequential delivery of cobalt and bone morphogenetic protein-2 for synergistic bone regeneration, Acta Biomater. 23 (2015) 295–308.
[79] M. Hu, M. Kurisawa, R. Deng, C.M. Teo, A. Schumacher, Y.X. Thong, L. Wang, K.M. Schumacher, J.Y. Ying, Cell immobilization in gelatin-hydroxyphenylpropionic acid hydrogel fibers, Biomaterials 30 (2009) 3523–3531.
[80] J.S. Im, J. Yun, Y.M. Lim, H.I. Kim, Y.S. Lee, Fluorination of electrospun hydrogel fibers for a controlled release drug delivery system, Acta Biomater. 6 (2010) 102–109.
[81] L. Wang, P. Wang, M.D. Weir, M.A. Reynolds, L. Zhao, H.H. Xu, Hydrogel fibers encapsulating human stem cells in an injectable calcium phosphate scaffold for bone tissue engineering, Biomed. Mater. 11 (2016), 065008.
[82] K. Lee, E.A. Silva, D.J. Mooney, Growth factor delivery-based tissue engineering: general approaches and a review of recent developments, J. R. Soc. Interface 8 (2011) 153–170.
[83] J.K. Tessmar, A.M. Gopferich, Matrices and scaffolds for protein delivery in tissue engineering, Adv. Drug Deliv. Rev. 59 (2007) 274–291.
[84] Y.R. Yun, J.H. Jang, E. Jeon, W. Kang, S. Lee, J.E. Won, H.W. Kim, I. Wall, Administration of growth factors for bone regeneration, Regen. Med. 7 (2012) 369–385.
[85] Z. Bai, X.H. Guo, C. Tang, S.T. Yue, L. Shi, B. Qiang, Effects of artesunate on the expressions of insulin-like growth Factor-1, osteopontin and C-Telopeptides of type II collagen in a rat model of osteoarthritis, Pharmacology 101 (2018) 1–8.
[86] J. Street, M. Bao, S. Bunting, F.V. Peale, N. Ferrara, H. Steinmetz, J. Hoeffel, J.L. Cleland, A. Daugherty, N. van Bruggen, Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover, Proc. Natl. Acad. Sci. Unit. States Am. 99 (2002) 9656–9661.
[87] L. Mi, H. Liu, Y. Gao, H. Miao, J. Ruan, Injectable nanoparticles/hydrogels composite as sustained release system with stromal cell-derived factor-1alpha for calvarial bone regeneration, Int. J. Biol. Macromol. 101 (2017) 341–347.
[88] M. Fujioka-Kobayashi, M.S. Ota, A. Shimoda, K. Nakahama, K. Akiyoshi, Y. Miyamoto, S. Iseki, Cholesteryl group- and acryloyl group-bearing pullulan nanogel to deliver BMP2 and FGF18 for bone tissue engineering, Biomaterials 33 (2012) 7613–7620.
[89] J.N. Zara, R.K. Siu, X. Zhang, J. Shen, R. Ngo, M. Lee, W. Li, M. Chiang, J. Chung, J. Kwak, B.M. Wu, K. Ting, C. Soo, High doses of bone morphogenetic protein 2 induce structurally abnormal bone and inflammation in vivo, Tissue Eng. 17 (2011) 1389–1399.
[90] A. Moshaverinia, C. Chen, K. Akiyama, X. Xu, W.W. Chee, S.R. Schricker, S. Shi, Encapsulated dental-derived mesenchymal stem cells in an injectable and biodegradable scaffold for applications in bone tissue engineering, J. Biomed. Mater. Res. 101 (2013) 3285–3294.
[91] A. Shekaran, J.R. Garcia, A.Y. Clark, T.E. Kavanaugh, A.S. Lin, R.E. Guldberg, A.J. Garcia, Bone regeneration using an alpha 2 beta 1 integrin-specific hydrogel as a BMP-2 delivery vehicle, Biomaterials 35 (2014) 5453–5461.
[92] T. Shirakura, C. Smith, T.J.J. Hopkins, Y.E. Koo Lee, F. Lazaridis, P. Argyrakis, R. Kopelman, Matrix density engineering of hydrogel nanoparticles with simulation-guided synthesis for tuning drug release and cellular uptake, ACS Omega 2 (2017) 3380–3389.
[93] B.B. Seo, J.T. Koh, S.C. Song, Tuning physical properties and BMP-2 release rates of injectable hydrogel systems for an optimal bone regeneration effect, Biomaterials 122 (2017) 91–104.
[94] J.L. Holloway, H. Ma, R. Rai, J.A. Burdick, Modulating hydrogel crosslink density and degradation to control bone morphogenetic protein delivery and in vivo bone formation, J. Contr. Release 191 (2014) 63–70.
[95] C. Tremolada, G. Palmieri, C. Ricordi, Adipocyte transplantation and stem cells: plastic surgery meets regenerative medicine, Cell Transplant. 19 (2010) 1217–1223.
[96] Z. Li, Z. Han, J.C. Wu, Transplantation of human embryonic stem cell-derived endothelial cells for vascular diseases, J. Cell. Biochem. 106 (2009) 194–199.
[97] J.M. Gimble, A.J. Katz, B.A. Bunnell, Adipose-derived stem cells for regenerative medicine, Circ. Res. 100 (2007) 1249–1260.
[98] S. Kern, H. Eichler, J. Stoeve, H. Kluter, K. Bieback, Comparative analysis of

mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue, Stem Cell. 24 (2006) 1294–1301.
[99] E. Jones, X. Yang, Mesenchymal stem cells and bone regeneration: current status, Injury 42 (2011) 562–568.
[100] L. Zhao, M.D. Weir, H.H. Xu, An injectable calcium phosphate-alginate hydrogel-umbilical cord mesenchymal stem cell paste for bone tissue engineering, Biomaterials 31 (2010) 6502–6510.
[101] P. Niemeyer, K. Fechner, S. Milz, W. Richter, N.P. Suedkamp, A.T. Mehlhorn, S. Pearce, P. Kasten, Comparison of mesenchymal stem cells from bone marrow and adipose tissue for bone regeneration in a critical size defect of the sheep tibia and the influence of platelet-rich plasma, Biomaterials 31 (2010) 3572–3579.
[102] M. Agung, M. Ochi, S. Yanada, N. Adachi, Y. Izuta, T. Yamasaki, K. Toda, Mobilization of bone marrow-derived mesenchymal stem cells into the injured tissues after intraarticular injection and their contribution to tissue regeneration, Knee Surg. Sports Traumatol. Arthrosc. 14 (2006) 1307–1314.
[103] J.A. Burdick, R.L. Mauck, S. Gerecht, To serve and protect: hydrogels to improve stem cell-based therapies, Cell Stem Cell 18 (2016) 13–15.
[104] X. Zhao, S. Liu, L. Yildirimer, H. Zhao, R. Ding, H. Wang, W. Cui, D. Weitz, Injectable stem cell-laden photocrosslinkable microspheres fabricated using microfluidics for rapid generation of osteogenic tissue constructs, Adv. Funct. Mater. 26 (2016) 2809–2819.
[105] N. Huebsch, E. Lippens, K. Lee, M. Mehta, S.T. Koshy, M.C. Darnell, R.M. Desai, C.M. Madl, M. Xu, X. Zhao, O. Chaudhuri, C. Verbeke, W.S. Kim, K. Alim, A. Mammoto, D.E. Ingber, G.N. Duda, D.J. Mooney, Matrix elasticity of void-forming hydrogels controls transplanted-stem-cell-mediated bone formation, Nat. Mater. 14 (2015) 1269–1277.
[106] A.J. Engler, S. Sen, H.L. Sweeney, D.E. Discher, Matrix elasticity directs stem cell lineage specification, Cell 126 (2006) 677–689.
[107] N. Huebsch, P.R. Arany, A.S. Mao, D. Shvartsman, O.A. Ali, S.A. Bencherif, J. Rivera-Feliciano, D.J. Mooney, Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate, Nat. Mater. 9 (2010) 518–526.
[108] O. Chaudhuri, L. Gu, D. Klumpers, M. Darnell, S.A. Bencherif, J.C. Weaver, N. Huebsch, H.P. Lee, E. Lippens, G.N. Duda, D.J. Mooney, Hydrogels with tunable stress relaxation regulate stem cell fate and activity, Nat. Mater. 15 (2016) 326–334.
[109] D.S. Benoit, M.P. Schwartz, A.R. Durney, K.S. Anseth, Small functional groups for controlled differentiation of hydrogel-encapsulated human mesenchymal stem cells, Nat. Mater. 7 (2008) 816–823.
[110] B. Baroli, From natural bone grafts to tissue engineering therapeutics: brainstorming on pharmaceutical formulative requirements and challenges, J. Pharmaceut. Sci. 98 (2009) 1317–1375.
[111] A.R. Short, D. Koralla, A. Deshmukh, B. Wissel, B. Stocker, M. Calhoun, D. Dean, J.O. Winter, Hydrogels that allow and facilitate bone repair, remodeling, and regeneration, J. Mater. Chem. B 3 (2015) 7818–7830.
[112] Y.J. Chuah, Y. Peck, J.E.J. Lau, H.T. Heec, D.A. Wang, Hydrogel based cartilaginous tissue regeneration: recent insights and technologies, Biomater. Sci. 5 (2017) 613–631.
[113] T.R. Hoare, D.S. Kohane, Hydrogels in drug delivery: progress and challenges, Polymer 49 (2008) 1993–2007.
[114] T. Yung-Hao, J. Khoneisser, H. Ping-Chun, X. Xiaoyang, Hydrogel as a bioactive material to regulate stem cell fate, Bioact. Mater. (Netherlands) 1 (2016) 39–55.
[115] B. He, Y.S. Ou, A. Zhou, S. Chen, W.K. Zhao, J.Q. Zhao, H. Li, Y. Zhu, Z.H. Zhao, D.M. Jiang, Functionalized D-form self-assembling peptide hydrogels for bone regeneration, Drug Des. Dev. Ther. 10 (2016) 1379–1388.
[116] P. Ni, Q. Ding, M. Fan, J. Liao, Z. Qian, J. Luo, X. Li, F. Luo, Z. Yang, Y. Wei, Injectable thermosensitive PEG-PCL-PEG hydrogel/acellular bone matrix composite for bone regeneration in cranial defects, Biomaterials 35 (2014) 236–248.
[117] A.H. Milani, A.J. Freemont, J.A. Hoyland, D.J. Adlam, B.R. Saunders, Injectable doubly cross-linked microgels for improving the mechanical properties of degenerated intervertebral discs, Biomacromolecules 13 (2012) 2793–2801.
[118] F. Anjum, P.S. Lienemann, S. Metzger, J. Biernaskie, M.S. Kallos, M. Ehrbar, Enzyme responsive GAG-based natural-synthetic hybrid hydrogel for tunable growth factor delivery and stem cell differentiation, Biomaterials 87 (2016) 104–117.
[119] F. Yang, J. Wang, L.Y. Cao, R. Chen, L.J. Tang, C.S. Liu, Injectable and redox-responsive hydrogel with adaptive degradation rate for bone regeneration, J. Mater. Chem. B 2 (2014) 295–304.