Materials for bone regeneration: Current types, bioactive mechanism and updated investigations

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Abstract

The exploration of bone repair materials has been continuously carried out. After several decades of development, bone repair materials have experienced from traditional ceramics, metals and polymers to modern smart hydrogels and multifunctional composite materials, *etc*. At the same time of designing and preparing the novel materials, more and more attention has been paid to their osteogenesis mechanism and immune response. The development of next generation of bone repair materials requires the comprehensive consideration of a combination of many related factors. This article reviews the research status and the progress of bone repair materials from the three aspects, current most-used types, bioactive mechanism and updated related investigations. Finally, several research points that are crucial for the further development of bone repairs are proposed in the conclusion and perspectives part.

Introduction

Bone defects beyond critical-size due to the injury, tumor or osteomyelitis, which affect revascularization and tissue regeneration directly, may not be repaired without external interventions. Nearly 4 million bone fractures happened in US in 2013. Due to the ageing population, by 2025, the global costs of osteoporotic fracture are expected to increase by 25%.[1] Another statistics states that in 2015, around 7 million people in America underwent total hip or knee replacement.[2] Bone repair materials have huge market prospect.

Among all bone substitutions, autologous bone grafts are still considered as the *gold standard*, but the size and source limit its application, as well as the risk of infection and ongoing unendurable pain after a second surgery at the donor site. Allograft is an alternative to autografts, but it may be immunogenic or have the risk of viral transmission, which can lead to a high failure rate. On this occasion, available natural bone grafts are far from satisfying the clinical requirements.

Based on these facts, ceramics, synthetic or natural polymer materials, metals *etc*. are commonly used as bone repair materials with large supplement and less or free immunogenicity. They may not only provide structural support, but also have a promoting effect on bone regeneration, which is effective to repair large bone defects. It was suggested that bone repair materials should have the ability to promote osteogenic differentiation of hMSCS or the proliferation of osteoblasts,[3] and ideal scaffolds for bone tissue engineering needed to have interconnected pores, appropriate porosity as well as suitable mechanical properties. Some studies emphasized on the ability to induce and support angiogenesis.[4] A suitable pore size was necessary for blood vessel growth, transport of nutrients and metabolic waste.

This review outlines the current types of bone repair materials, from the ceramic, metal and polymer materials, carbon materials, to the smart hydrogels, multifunctional composite materials and natural biological material *etc*., bioactive mechanisms involved and recent investigations, including functional improvements and immunological researches. Finally, this review analyses the future developments of bone repair materials.

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Key words: bone repair material; bone tissue engineering; bioactive mechanism; immune response.

Contributions: YH provided the original drafts of the paper and editing; all other authors contributed to the design of the paper framework and concepts; XL provided integral guidance, detailed manuscript editing, and provided the foundations and final approval of the version to be published.

Conflict of interest: the authors declare no potential conflict of interest.

Funding: the authors acknowledge the financial supports from the National Key R&D Program of China (No. 2017YFC1104703), National Natural Science Foundation of China (Nos. 31771042 and 11827803), Laboratory of New Ceramic and Fine Processing Tsinghua University (No. KF201714), International Joint Research Center of Aerospace Biotechnology and Medical Engineering, Ministry of Science and Technology of China, and the 111 Project (No. B13003).

Received for publication: 31 January 2019.
Accepted for publication: 14 February 2019.

BioMaterials Advances 2019; 2:63
doi:10.4081/bioadv.2019.63

Current materials and their applications

At present, there are many types of bone repair materials, such as bioceramics, metals, polymers and composites. Besides, carbon materials are also widely used. Currently, smart hydrogels, metal ions doped composites and natural biomaterials *etc*. have been well developed. Some of these have made remarkable achievements in animal models or clinical researches.

Conventional materials

Bioceramics and bioactive glasses

Calcium phosphates (CAPs) and bioactive glasses are commonly used as bone repair materials. CAPs, including hydroxyapatite (HA), tricalcium phosphate (TCP) and biphasic calcium phosphate (BCP), have good biocompatibility and osteogenic activity.[5] It had been shown that the CAPs could form strong chemistry bonds with surrounding bone and promote osseointegration.[6-8]

HA is similar with the main component of natural bone,[9] and can bond with natural bones in body. With good osteogenic activity, it had been used as bone repair materials before century 21.[10] The surfaces of HA can not only provide nucleating sites for the precipitation of apatite crystals in culture medium,[11,12] but also accelerate apatite growth.[13] A study showed that HA could be slowly degraded by simple dissolution or by osteoclastic bone remodeling effect.[14] However, with low breaking strength and high brittleness, it may be unsuitable to be

directly used in large-scale bone defect repair. Fortunately, with better bioactivity and higher mechanical strength, nano-HA may be an ideal bone repair material.[15] For example, Kubasiewicz *et al*.[16] created 5 mm calvaria bone defects in rats and used nano-HA to repair. Histological analysis and micro-CT evaluation showed that 34.2-44.4% new bone was formed after 8 weeks, whereas only 13.0% in empty control group.

β-TCP, a good osteoconductive material, was reported to have similar effects to inorganic bovine bone (Bio-Oss, Geistlich Pharma, Wolhusen, Switzerland) in critical-size defect repair of rats.[17] For patients with tibial plateau fractures, a long-term study showed that new bone was formed around β-TCP with good functional recovery, which meant that β-TCP was an effective material in tibial plateau fracture treatments.[18]

BCP ceramic, a mixture of HA and TCP, has been widely used in clinical. It is reported that the BCP might be a feasible alternative to autografts.[19-23] In the surgical management of scoliosis, the correction was maintained similarly in BCP and autografts groups, but BCP groups had lower blood loss and free postoperative local complications.[20] And in another study, BCP could achieve equivalent fusion rates to autograft in rabbit lumbar arthrodesis models.[21] In addition, Fellah *et al*.[23] compared the osteogenicity of BCP granules with autograft, the results of which showed that the BCP had better osteogenic property and stability in critical-sized bone defect repair.

Bioactive glasses are biodegradable in physiological conditions. They can release ions, such as Na^+ and Ca^{2+}, to give rise to new bone regeneration with mechanically strong bond to the surfaces of implanted.[24] They have osteoconductivity, and can bond firmly both with natural bones and soft tissues. However, high brittleness limits their applications to repair load-bearing bones.[25] So, it is pressing to optimize bioactive glasses by innovative scaffold design and processing.

Metals and their alloys

As early as last century, due to high mechanical strength, metals and their alloys have been widely used for load-bearing applications in bone repair, including bone pins, plates, screws, knee or hip prostheses and dental implants, *etc*.[26] Alloys, which generally perform better than pure metals, are more commonly used. Stainless steel, Co-based alloys and Ti-based alloys are the three main types for bone repair.

With low cost and good processibility, stainless steel, such as 316L stainless steel, is still widely used for implant devices today. However, many studies had shown that the stainless steel often underwent corrosion failure in the body[27] or in simulated body fluids,[28] especially with the existence of Cl^-.[29] As a matter of fact, stainless steels are mostly used for temporary implant devices.[26] Co-based alloys, like Co-Cr and Co-Ni, have been made for hip joint prostheses or other devices with fine-structures.[26] They display high fatigue strength and wear resistance. It was reported that Co-based alloys had better corrosion resistance compared to others.[30] However, it was reported that in long period implantations, a high amount of released Cr and Ni ions might lead to negative toxic effects[31] or allergic responses,[32] *etc*. Moreover, Ti is a suitable option for bone repair with superior biocompatibility, low density (4.5g/cm^3), good corrosion resistance, low elastic modulus, and non-toxic even in large doses.[29] Researches showed that Ti and Ti-based alloys could make good physical connections with the host bone[30] and had been widely used in clinical, such as total hip replacements and craniofacial implants.[26] However, there are some common problems of metals and their alloys, such as the stress shielding effect due to the mismatch between elastic modulus of metal implants and that of natural bone, corrosion and fatigue fractures, inflammatory reactions, and allergic reactions caused by the wear debris or metal ions. One promising strategy to achieve suitable elastic modulus is to prepare porous metallic implants. It is also needed to control the corrosion rate to avoid metal-related toxicity by precise modification and control of micro- and nano-structures.

Polymers

Polymers can be divided into natural and synthetic ones. Natural materials involve collagen, silk fibroin (SF), chitin (or chitosan), hyaluronic acid, sodium alginate, *etc*. Synthetics mainly include polylactic acid (PLA), Polycaprolactone (PCL) and polylactic acid-glycolic acid copolymer (PLGA) and so on.

Collagen is the main organic component in natural bone and serves as the template for mineralization with specific structures and bioactivities. However, pure collagen is unsuitable for direct applications due to its poor mechanical strength and high degradation rate. Fortunately, reports showed that it could encapsulate the MSCs as injectable microspheres for bone repair,[33] or combine with bioceramics or other polymers to further increase the mechanical and biological properties.[34]

SF is a kind of natural biopolymer obtained from spiders or silkworms silks.[35] With satisfactory flexibility, extensibility and tensile strength, it can be molded into different kinds of forms.[36] It can offer active sites to aid mineralization or bond with bioactive molecules to promote bone regeneration. It was reported that the electrospun silk fibroin scaffolds could facilitate the new bone formation in rat calvaria defect models more significantly than PLA scaffolds.[37] Moreover, the degradation rate of silk fibroin could be controllable, ranging from weeks to a year.[38,39] So, SF is expected to be widely applied to repair bone in the phase of human trials soon.[40] With varied mechanical properties and processability, PLA is widely used for bone repair in clinical.[41] Up to now, there are many types of biodegradable PLA devices, such as screws, plates and pins, which, unlike alloys, can not only withstand bear-loading and transfer stress to the damaged area to avoid stress shielding effect over a period of time, but also avoid a second surgical procedure to remove. However, they have some weaknesses, such as poor hydrophilicity and excessively rapid degradation rate, *etc*. Moreover, the accumulation of the degradation product, lactic acid, *in vivo* might lead to a too low local pH, which could cause inflammatory reaction[42] or even osteolysis.[43,44] Therefore, to neutralize the acidity or to modify properties, PLA was often composite with other fillers, such as HA, chitosan.[45]

In short, both natural and synthetic materials have their own advantages and disadvantages. For example, on the one hand, most natural ones have low immunogenicity, no cytotoxicity, good biocompatibility and bioactivity. Some of those have high mechanical properties, osteoconductivity or osteoinductivity. Besides, the structures of some natural materials are similar to those of the natural bone ECM. However, some natural materials are difficult to obtain or process,[46] and sometimes the performance may vary with batches. Furthermore, chemical or enzymatic treatment may damage their functionality or even result in inactivation. On the other hand, synthetics have well-adjustable mechanical properties and outstanding processability, which can be easily modified into various shapes or to mimic natural matrices. But some have poor biocompatibility, too rapid or slow degradation rate, less integration with the surrounding tissue.

Composites

A single material often has inevitable shortcomings, so people turn their attention to the composites, which may combine the positive functionalities of each component

and compensate the disadvantages of the individual or even have new properties that are not available in either material used alone.[47]

Polymers, ceramics and metals can be used as matrixes or reinforcements for bone repair composites to acquire better properties. For example, Li *et al*.[48] prepared nano-HA/collagen/PLLA composites reinforced by chitin fibers, which had higher compressive strength than nano-HA and were similar to natural bone in components with the existence of the chitin fibers. The further study *in vivo* showed that the composites could repair 40 mm goat shank bone defect successfully in 15 weeks, which is firstly reported to show that the use of biomaterials repaired completely segmental bone defects larger than 30 mm.

In addition, Chen *et al.*[49] prepared and investigated the collagen-SF/HA nanocomposites, which had suitable structural properties, good biocompatibility *in vitro* and an enhanced elastic modulus with the presence of SF in comparison to collagen/HA composites. In another study, Zhang *et al*. developed small intestinal submucosa/polymethyl methacrylate (SIS/PMMA) composites for vertebral repair in rats. With a higher porosity, decreased stiffness and greater osteoinductivity, they could enhance the adhesion, proliferation and osteogenic differentiation of MC3T3-E1 cells and BMSCs compared to PMMA. And further study indicated that these composites can greatly enhanced osteointegration and bone regeneration in vertebral defect models.[50] In this system, PMMA serves as a basic material with stiffness and formability, and the bioactive SIS enhanced the biological performance. Moreover, it was reported that the PLGA/TCP porous scaffold with Mg ions incorporated had good bioactivity and enhanced mechanical properties. As the released of Mg ions in this system raised the activity of osteoblasts, this scaffold could promote more new bone formation than PLGA/TCP scaffold in 15 mm radius defects of rabbits.[51]

Carbon nanomaterials

With excellent mechanical properties and biocompatibility, carbon materials, especially carbon nanotubes (CNTS) and grapheme, have attracted more and more attention.

CNTs are cylindrical nanostructures and have extraordinary properties, like good tensile strength, high aspect ratio[52] and good biocompatibility *in vivo*.[53,54] Generally, they are often used as reinforcing nanofillers in bone repair composites. It was reported that the stiffness of CNT-ploymer composites could be increased by 7 times.[52] Another research showed that the HA-CNTs composite had considerable improvement in fracture toughness and flexural strength over pure HA, and the toughness of this composite was close to that of human bone. Further study proved that it could enhance new bone formation with strong interactions and high interfacial strength with the host bone in rabbit models.[55] Though some studies showed that carbon nanotubes were poor in hydrophilicity and easy to be agglomerated, they could be functionalized[56] or modified on the surface to remedy the weaknesses. For example, Venkatesan *et al*.[57] fabricated chitosan-MWNTs composites for bone repair. This bicomponent system had increased porosity and could enhance cells proliferation and ALP expression than chitosan scaffold. It was also demonstrated that after carboxyl-functionalization, the MWNTs were uniformly distributed with the chemical interactions formed with chitosan. Besides, it had been reported that the specific treated multi-walled carbon nanotubes (MWNTs) could absorb bone-formed related proteins to promote stem cells differentiate into osteoblasts.[58,59]

Another promising carbon nanomaterial, grapheme, is an allotrope of carbon with a single atomic layer of six-atom rings network, which has many unusual properties, such as large specific surface area,[60] distinguished electrical conductivity and mechanical properties.[61] Many studies showed that grapheme had potential good biological property and could improve the adhesion, proliferation and differentiation of osteoblasts,[62] which indicated that it had marvelous potential in bone repair. However, there are still some shortcomings of graphene, such as the long-term toxivcity and poor biodegradability.[63] So, its applications in bone repair still remain studies *in vitro* or animal experiments stages.

Smart hydrogels

Although the concept of hydrogels appeared at the end of the 19th century, the word *smart* was not introduced until 1948 by Kuhn.[64] The smart hydrogels can not only present specific structures and functions by one or more stimuli, like temperatures, pH, electric, magnetic fields, light and biomolecule, but also be customized for different targets, such as chronic inflammation treatment, cancer therapy and bone repair.[65] Nowadays, smart hydrogels have become research hotspots in bone repair. The number of publications in the Science Direct database about smart hydrogels and smart hydrogels for bone repair since 1998 has had a booming growth (Figure 1).

It has been showed that the release rate of the bioactive proteins, cells or drugs loaded in smart hydrogels with three-dimensional structures could be controlled by different external stimuli,[66,67] thereby providing potentials as intelligent drug delivery systems in a controlled manner for bone repair applications. Papathanasiou *et al*.[68] prepared the programmable and responsive silica-based hydrogels, with bisphosphonates (BPs) loaded, for osteoporosis therapy. The release rate of BPs could be

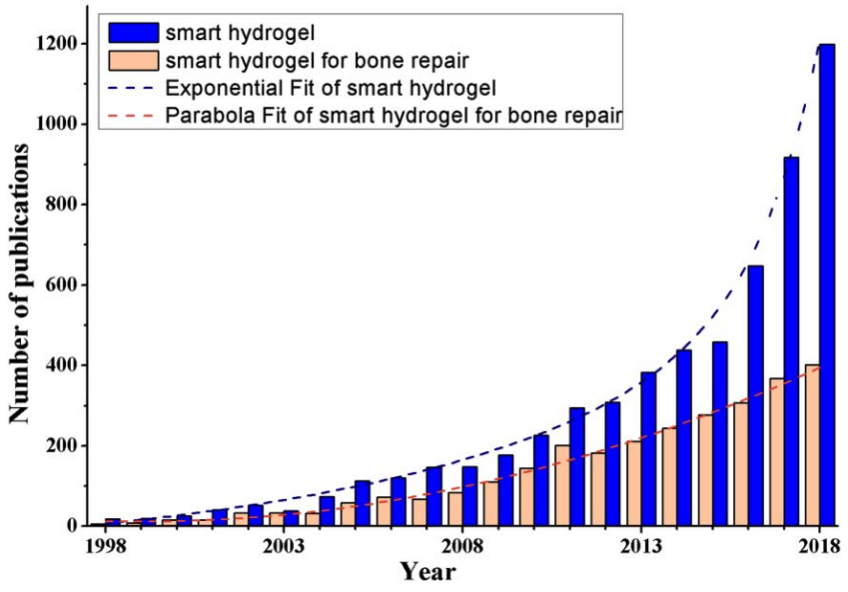

Figure 1. The number of publications about smart hydrogels or smart hydrogels for bone repair in Science Direct database per year. (Compiled from a literature search in Science Direct database in December 2018).

fine-tuned by several factors, like cations, pH, temperature and side-chains of BPs, to meet the patient's requirements. For example, the change of pH could reinforce or weaken the binding strength of hydrogen bonds in the hydrogels to affect the gelation to control the release rate of BPs. Moreover, injectable smart hydrogels can perfectly fit defects of different sizes and irregular shapes with homogenous distribution, minimal invasion and low risk of infection. They can perform good integrations with host bone and enhance new bone formation. For example, one kind of reported injectable acellular bone matrix/PEG–PCL–PEG hydrogels were bioactive and thermosensitive, which could be molded into the shape of the defects perfectly and gel in a few minutes at body temperature in cranial defects of rabbits. The X-ray examination, micro-CT images and histological analysis all proved that these hydrogels could be effective in cranial defects repair, and the density of new formed bone was approximate to that of the host cranial bone after 20 weeks.[69] Furthermore, it is well known that dynamically tunable cell culture platforms are similar to natural cell microenvironments. Based on their stimuli-responsiveness to pH, temperature, radiation, *etc*., smart hydrogels can provide various dynamic chemical, physical, and mechano-structural cues to the adhered cells in the platforms, where the fundamental biological processes can be deeply understood, and the interactions between implants and cells can be well simulated.[70] The smart hydrogels in these platforms can present different mechanical properties to simulate the *in vivo* ECM to control cellular processes. For example, Yang *et al*. developed a phototunable PEG hydrogels with tunable stiffness to stimulate the highly dynamic ECM, which could be used to investigate the response of hMSCs to the stiffness of the substrate and whether the MSCs had the memory of the previous mechanical signals.[71] With significant advantages, smart hydrogels are commonly used in bone repair and delivery system.[72] But, it is still needed to consider about controlled biodegradability and the maintenance of mechanical properties after implantation.

Metal ions

Natural bone microenvironment contains a variety of bioactive metal ions, such as Strontium, Zinc and Magnesium ions, which often exist in many surrounding macromolecules, like enzymes and nucleic acids, *etc*. It has been reported that some metal ions could induce bone related gene expression and bone metabolism via signal transduction to enhancing osteogenic differentiation of cells, osteogenesis and angiogenesis.[73] Therefore, with the adoption of some specific metal ions, bone repair materials have potentials to exhibit better performances. Most of the Strontium (Sr) element in the human body exists in the skeletal system,[74] which has been proved not only to stimulate new bone formation,[75] but also to inhibit bone resorption.[76] For example, it was reported that the addition of Sr into HA could improve the adhesion, viability and ALP activity of MG-63 cells, indicating that the SrHA composites had good potential to promote bone growth.[77] Moreover, Prabha *et al*. prepared the poly-(ε) caprolactone-laponite-strontium ranelate (PLS3) scaffold for bone tissue engineering, which had shown that the PLS3 was osteoinductive and could support the proliferation and osteogenic differentiation of hMSCs.[78] After seeded with hMSCs, it could promote ectopic bone formation with vascularization in immunocompromised mice, and the strontium ranelate in this system could enhance the density of the new formed bone at the implant site.

Magnesium (Mg) ions are bio-safe both *in vitro* and *in vivo*, and can be promptly diluted and excreted.[79] They exist in natural bone and can stimulate bones growth[80-82] or remodeling.[83] Mg ions perform as cofactors in various bone-related enzymatic reactions, such as protein and nucleic acid synthesis, energy metabolism and vitamin D metabolism.[84] As mentioned above,[51] PLGA/TCP porous scaffold incorporated with Mg ions could promote osteogenesis. Another study showed that the presence of Mg in TCP scaffolds could facilitate angiogenesis in femoral defect of rat models.[85]

Zinc (Zn) ions are also necessary in protein synthesis, cell proliferation and DNA synthesis, which can stimulate bone formation and inhibit bone resorption. For example, Bhattacharjee *et al*.[86] doped Zn ions in HA to improve its bioactivity. After the implantation in femur bone of rabbits for two months, the percentage of new bone formation in Zn-HA group was significantly higher than that of pure HA group. Histological studies showed that well-development of Haversian system and Volkmann's canal was formed in the regenerated bone. Moreover, mechanical push-out testing demonstrated stronger interfacial strength between the Zn-doped implants and host bone. Another study showed that in Zn-doped TCP cement, the low concentration of Zn^{2+} could promote the proliferation and ALP activity of rat MSCs to enhance bone formation.[87] Furthermore, the Zn^{2+} could offer anti-bacterial property to TCP to prevent clinical infections. However, high concentration of Zn^{2+} might restrict cell growth,[88,89] so it is necessary to control the amount of Zn^{2+} in the composites and their release rate.

In fact, the current use of metal ions in bone repair materials mostly stays in the stage of animal experiments only with few clinical results, so further studies on the doping methods and precise release of metal ions are necessary.

Natural biomaterials

With specific advantages, such as low immunogenicity and similar structure to that in the human body, natural biomaterials for bone repair have attracted much attention. Among all, those derived from aquatic organisms, like corals and fish collagens, with a wide range of source, good bioactivity and osteogenic activity, have good potential for bone repair. As early as the 1980s, corals had been investigated and used as bone repair materials.[90] Nowadays, there are some effective commercially available products in clinical, like Pro Osteon™ and Biocoral®.[91] The structures of corals were networks with interconnected channels and pores, which were highly similar to those of cancellous bones and could allow for the ingrowth of capillaries.[92] Besides, it had been proved that hMSCs and osteoblasts could adhere, proliferate or differentiate on the coral scaffolds with high DNA content and ALP activity.[93,94] Nevertheless, in some other studies, corals had relatively low resorption and fusion rate in cervical arthrodesis,[95-97] and poor bone ingrowth at the defects of iliac crest,[98] which indicated that the experimental results were inconsistent and more in-depth investigations into their long-term performances were needed. Moreover, the overexploitation and environmental changes had put them in an endangered position.[94] Meanwhile, corals might contain some toxic elements due to the environment pollution.[99] So, it is necessary to launch better protection measures and detailed examinations into corals before considering the application to bone repair, or try to cultivate artificial corals. Due to its wide range of sources, low cost and minimal disease transmission,[100] fish collagen is considered as an appropriate alternative to mammal collagen for bone repair. It was reported that the bioactive tilapia collagen could increase the expression of ALP, osteocalcin and osteopontin in pre-osteoblasts *in vitro*.[101] Moreover, Matsumoto *et al*. demonstrated that the flexible and soft tilapia collagen could induce the adhesion and the early osteoblastic differentiation of hMSCs better than the hard porcine collagen. And the

aligned tilapia collagen fibrils could induce cell polarization and facilitate osteogenesis while the randomly arranged porcine collagen didn't have similar functions.[102] However, it was showed that fish collagen might be allergens for some people.[103] Therefore, more hyposensitization and detailed safety tests of fish collagen should be taken into consideration.

Bioactive mechanism of the materials

Generally, there had been many studies on the approaches for designing the bone repair materials and the expression of bone-related gene or proteins, but the intrinsic bioactive mechanisms were often ignored.[104,105] Fortunately, more and more recent studies have begun to focus on their bioactive mechanisms. Nowadays, it has been shown that bone repair materials exhibit their functions by affecting signaling pathways of osteogenic-related cells, such as Notch, Wnt and BMP[106] (Figure 2). For example, Jung *et al*. reported that the silk fibroins could up-regulate the expression of ALP and Runx2 mRNA by inhibiting Notch signaling pathway of bone marrow cells to regulate osteogenesis.[107] Moreover, studies showed that, by significantly up-regulating the activity of BMP2[108] and Wnt[109] signaling pathways of MSCs, the β-TCP could stimulate the bone regeneration. In another study, Zhang *et al*. showed that Mg^{2+} could increase the expression level of Runx2 and ALP to significantly improve the osteogenic activity via TRPM7/PI3K signaling pathway of human osteoblast cells.[110] Besides, some composites have been shown to promote bone repair with the similar mechanism. For example, Liu *et al*. had demonstrated that the nano-HA/chitosan scaffold could activate the integrin-BMP/Smad signaling pathway to up-regulate the mRNA expression level of the BMP-2/4, Runx2 and ALP to induce the osteogenic differentiation of mBMSCs.[111] Meanwhile, the physical and chemical properties of materials have effects on signaling pathways. For example, studies had proven that the surface micro/nanotopography of materials could influence the Hedgehog-Gli1,[112] FAK-ERK1/2,[113] ILK/ERK1/2 and ILK/p38[114] signaling pathways to regulate cell fates. In addition, it had been found that the increasing matrix stiffness could activate the Wnt signaling pathway to promote the proliferation and osteogenic differentiation of dental pulp stem cells,[115] or could up-regulate the expression of macrophage migration

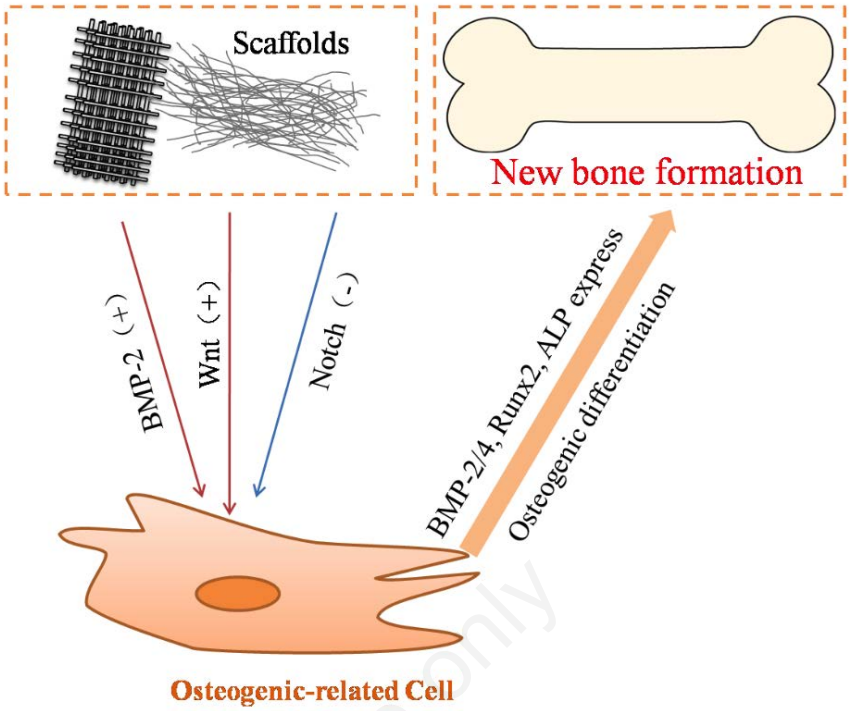

Figure 2. The bone repair materials can promote cell proliferation and differentiation through affecting the Notch, Wnt and BMP-2 signaling pathways to stimulate the expression of BMP-2/4, Runx2 or ALP, etc. in osteogenic-related cells to promote new bone formation.

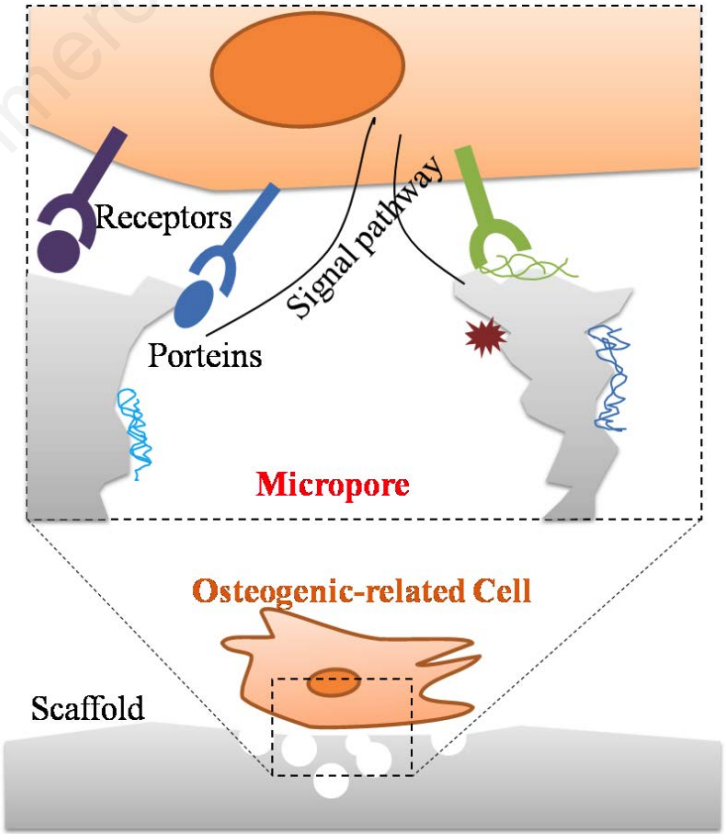

Figure 3. The porous scaffold can adsorb different proteins *in vitro* to promote cell adhesion and proliferation (the walls of pores are showed in grey).

inhibitory factor (MMIF) to activate the AKT/YAP/RUNX2 signaling pathway to facilitate osteogenic differentiation of hMSCs.[116] Furthermore, the porous nanomaterials could provide adsorption sites to gather and accumulate a large amount of bone-related proteins from the culture medium to promote cell adhesion and proliferation (Figure 3).[117-119] Moreover, the recruitment of endogenous MSCs *in vivo* is critical to bone regeneration. It had been hypothesized that MSCs might migrate to the targeted sites due to the participation of chemokines, growth factors, or other chemical or physical cues from bone repair materials. For example, it was reported that the bFGF-loaded acellular dermal matrix (ADM) could recruit a plenty of MSCs to the implant sites and promote their proliferation to a sufficient amount to facilitate bone regeneration.[120] In another study, Shih *et al*. showed that the PEGDA-co-A6ACA hydrogels could promote the osteogenic differentiation of MSCs, ES and even iPSCs without the addition of growth factors. After the subcutaneous implantation in the spine of rats, it could recruit endogenous progenitor cells for bone formation by the simulation of the bone environment to assist spinal fusion.[121,122] In addition, Song *et al*. implanted the osteoinductive microporous BCP in dogs' dorsal muscles and observed that the osteoprogenitor cells and BMSCs were homed from the bone marrow to the implant site through blood circulation to induce ectopic bone formation.[123]

Updated investigations of bone repair materials

Nowadays, bone repair materials have been continually optimized to improve their biocompatibility, bioactivity and osteogenic capability, and to overcome the shortcomings. Furthermore, the immunoregulation effect of materials was also underlined in some studies.

Function improvement

Recently, with the development of manufacturing technology and the in-depth researches, more and more multifunctional bone repair materials have appeared. On the one hand, these materials not only have the function of the conventional materials, but only have suitable degradation rate, antibacterial property and the function to promote vascularization, *etc*. On the other hand, these conventional functions, bioactivity and osteogenesis ability, *etc*. had been further improved.

Multi-function

Much attention has been paid to the multifunctionality of bone repair materials to obtain better repair efficiency. For example, in a recent study of Li *et al*., with the reinforcement of silica nanoparticles (SNs), the biodegradable and bioactive poly (citrate-siloxane) had the enhanced photoluminescent capacity for imaging application. The further *in vitro* experiment showed that the osteoblasts (MC3T3-E1) had improved attachment and proliferation on this material. Therefore, it was indicated that this material had the potential to realize simultaneous bone regeneration and fluorescence imaging.[124] In another study, with Co ions incorporated, the bioactive glass-collagen-glycosaminoglycan scaffold could support osteogenesis and promote the expression of VEGF, indicating that this scaffold has both pro-angiogenic and pro-osteogenic capabilities for bone repair.[125]

Bioactivity improvement

In order to further improve the bioactivity and osteogenesis ability of materials, bioactive molecules, like peptides and growth factors, have been adopted into the bone repair materials.

Recently, osteogenesis-related polypeptides have been widely used in bone repair materials due to their outstanding osteogenic ability, low cost, stability and minimal tumor-related side effects.[126] Generally, these polypeptides can be bonded to the surface of materials by either non-covalent or covalent bonds. But the covalent combination can effectively increase the interactions between the polypeptides and materials, prolong the duration of polypeptides action and make the functionalized surfaces more stable than the non-covalent one.[127] It was reported that, many polypeptides could be added in bone repair materials to mimic native bone ECM. Among them, Arg-Gly-Asp (RGD) polypeptide, which could be found in fibronectin and other molecules in ECM, could be coated on bone repair materials to enhance the adhesion and differentiation of osteoprogenitor cells.[128]

Loading growth factors on bone repair materials is another common method to improve the osteogenesis ability. For example, Zhao *et al*. immobilized BMP-2 on the surfaces of PLGA/HA fibrous scaffolds to increase the expression of osteogenesis-related genes and up-regulate the activity of ALP *in vitro*.[129] In another study, Kuttappan *et al*. stated that the sustained local delivery of multiple growth factors might be a potential approach for bone repair.[130] They developed PLLA-nanoHA-gelatin composite scaffolds with dual growth factor (VEGF-BMP-2 or FGF-2-BMP-2) loaded, by dissolving these growth factors with specific ratio in phosphate buffered saline (PBS, pH 7.4). Further *in vivo* study showed that, during 24 h, about 23 % of BMP-2, 42% of FGF-2 and 85% of VEGF were released steadily. VEGF could be released totally within 7 days while BMP-2 and FGF-2 could be released till 20 days. Increased new vasculature in the scaffolds and new bone growing from the peripheral region towards the mid region of the defect were observed in calvarial defects of rats, the results of which demonstrated that these scaffolds could effectively promote vascularisation and bone regeneration.

Immune response

As foreign bodies, bone repair materials can inevitably cause immune responses after the implantation. The interactions between materials and the surrounding immune environment should be considered, as well as the assessment of the osteogenic capacity of bone repair materials. For example, in a research of Chen *et al*., hMSCs and macrophages were cultured alone or together on cobalt incorporated β-tricalcium phosphate (CCP). When hMSCs were cultured alone, the CCP had the effect to promote their osteogenic differentiation. But this effect was reduced with the presence of macrophages. After the implantation in femur of rats, CCP was coupled with fibrous encapsulation rather than promoted new bone regeneration. The research showed that the CCP could shift macrophages to an M1 phenotype and cause enhanced inflammation. Therefore, the excessive inflammation impeded the osteogenic differentiation.[131]

Furthermore, other studies reported that macrophages played key roles in the recruitment and differentiation of MSCs. It was a common understanding that the coordinated cross-talk between immunocyte and bone related cells, especially macrophages and MSCs, could contribute to successful bone healing.[132]

Moreover, it has been shown that materials with different surface properties might induce diverse types or extent of immune responses.[133] For example, the surface roughness and hydrophilicity could influence the interaction between immune cells and materials. Hotchkiss *et al*. showed that, by increasing the roughness and surface wettability, titanium implant could polarize the adaptive immune response towards a Th2, pro-wound healing phenotype, to lead to a faster resolution of inflammation, by which, this implant could recruit more MSCs to the implant site with the presence of macrophages.[134]

Conclusions

Although rapid developments on bone repair materials, there has not been a material that fully meets the ideal requirements, especially for large bone defect repair. Most materials remain in the *in vitro* or animal experiments. With the advancement of science and the improvement of people's living standards, the performance of bone repair materials is undoubtedly facing higher requirements and challenges.

Firstly, it is needed to produce bone repair materials that have multiple functions or can simulate biological processes. Materials are expected to accurately assist the natural healing process via additional functions. The treatment plan and the implanted material should be different for the bone defects caused by different reasons. For example, i) for trauma and infection, the materials should have antibacterial and anti-infective properties by adding chitosan, silver ions or loading drugs; ii) For bone tumor, after excision of the tumor, the cancer cells left at the edge of the defects will multiply and cause local recurrence. The implanted bone repair material should also have the ability to inactivate cancer cells to prevent recurrence while promoting bone regeneration; iii) For fractures due to osteoporosis, it is better that the implanted material can not only promote the homing of osteoblasts and stimulate bone regeneration in situ, but also release Ca^{2+} to increase the calcium content and bone mineral density of host bone, thereby reducing the rate of fracture recurrence.

Secondly, by structural adjustment, bone repair materials can be effectively combined with bioactive molecules and achieve stable and precise release for a sufficient period of time. It is a viable way to wrap or physically or chemically bind porous microspheres with bioactive molecules inside with bone repair materials. Another promising method for precise release is preparing multilayered scaffolds with different types or dose of growth factor. Besides, the materials may have the structure to adsorb specific growth factors. After implanted in the body, they can accumulate growth factors, and then release them gradually. Moreover, efforts to design materials with specific structure to stimulate related cells to express osteogenic proteins, and then adsorb the proteins on their surface to further bring positive effects to other attached cells, have been another promising research direction for bone repair materials.

Thirdly, in the wake of development in high technology, the approaches of processing and preparing materials are more diversified. Advance techniques, such as 3D printing, and even the emerging 4D printing,[135] can manufacture materials with more complex structures to satisfy precious requirements. 3D CT scan image can capture the sophisticated details of the defects accurately. By the conversion of 3D CT scan image into mathematical model, biomimetic customized implants can be manufactured accurately through 3D printing. These techniques make it promising to realize the personalized medicine.

Fourthly, after implanted, materials are in a dynamic biomechanical environment instead of static. However, most of the current *in vitro* studies often ignore the influence of biomechanics. For example, although many bone bioreactors have been developed *in vitro*, they still have limitations and cannot fully simulate the complex environment of living organ. In many cases, the rates of material degradation *in vivo* and *in vitro* are different because of biomechanical environment. Thus, more attention should be paid on the effect of mechanics during the investigations into the degradation of materials in simulated body fluids or co-culture with cells. The *in vivo* mechanical environment can be simulated by adding a near physiological load. It is necessary to systematically study the relationship between material structures, mechanical properties and degradation in biomechanical environment to optimize bone repair materials. Moreover, at present, most of the results obtained in animal experiments may not be repeated in clinical.[136] On the one hand, a material should be tested from small animals to large ones systematically. Generally, small animals, such as rats and rabbits, have fast bone turnover rates and low cost, which can be used to assess the performances of materials initially. The advantage of large animals, such as goats and pigs, is that their physiology and biomechanical properties are closer to those of human being. So, experiments from small to large animals can help to find the rules of the repair process. Based on the comparison of the results obtained from small animals with those from large animals, the performances in clinical can be predicted. On the other hand, at present, there is hardly clinical standard to evaluate the degree of bone regeneration in animal experiments and clinical. So, a well recognized material should be selected first. Then, systematical and comprehensive investigations on this material both in animal models and in clinical are performed. Based on the results, an industry standard can be established to evaluate performances of other materials in the subsequent studies.

Fifthly, it is well know that vascularization, which is essential for the transport of nutrients and the recruitment of cells of the implant materials, is a prerequisite for bone healing,[137] especially for large bone defect repair. Although there are a lot of related researches, some of which took pore size,[138] vascular-related growth factors and cell co-culture,[139] as well as hypoxia-induced signaling[140] into account. Cell sheet technology, pre-patterning and 3D printing strategies are also worth trying,[141] deeper understanding and study of the interactions between materials and endotheliocyte or related cells are necessary. Furthermore, it may be an effective solution to prepare bone repair containing some specific tubes loaded with vascular-associated growth factors.

Finally, bone repair materials with specific immunomodulatory functions should be another important future development direction.[131] Immune cells play important roles in regulating skeletal dynamics. After materials are implanted, the first cells that they encounter are immune types. The regulation of the immune cell response by the material determines to some content whether bone regeneration can be promoted. At present, it is necessary to launch a large number of systematical investigations into the effects of the structure, composition and surface properties, *etc*. of bone repair materials on the differentiation of immune cells.

In short, the preparation of suitable bone repair materials is required to consider the multiple effects of various factors. Every factor is crucial and complements each other. It is necessary to advance long-term, cross-discipline collaborations. Further studies on the structure and properties of the materials, the mechanisms of angiogenesis, immune response and bone regeneration are imperative. With the above comprehensive developments, it should be much easier to achieve the satisfactory bone repair materials to meet the requirements of different patients for precision medicine.

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