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REVIEW ARTICLE

Recent progress in injectable bone repair materials research

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ABSTRACT: Minimally invasive injectable self-setting materials are useful for bone repairs and for bone tissue regeneration *in situ*. Due to the potential advantages of these materials, such as causing minimal tissue injury, nearly no influence on blood supply, easy operation and negligible postoperative pain, they have shown great promises and successes in clinical applications. It has been proposed that an ideal injectable bone repair material should have features similar to that of natural bones, in terms of both the microstructure and the composition, so that it not only provides adequate stimulus to facilitate cell adhesion, proliferation and differentiation but also offers a satisfactory biological environment for new bone to grow at the implantation site. This article reviews the properties and applications of injectable bone repair materials, including those that are based on natural and synthetic polymers, calcium phosphate, calcium phosphate/polymer composites and calcium sulfate, to orthopedics and bone tissue repairs, as well as the progress made in biomimetic fabrication of injectable bone repair materials.

KEYWORDS: bone repair material; polymer; calcium phosphate; calcium sulfate; biomimetic

Contents

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1 Introduction

Bones can easily suffer from defects or damages caused by

osteoporosis, trauma, tumour, infection and other diseases, and this has become one of the most devastating problems in human health [1]. Traditional therapies used for bone repairs rely on the implantation of bone grafts, which involves the shaping of corresponding bone substitutes *in vitro* and then their implantation through a surgical procedure. Despite the satisfactory results that they provide, these therapies can cause increased bone loss, trauma for the surrounding tissues, and large surgical wounds. A potential improvement over the traditional therapies is to repair bones using an injectable material. This type of materials can be injected into the damaged tissues by an invasive technique and then mold to the shape of the bone cavity *in situ* to set and fill in the defects. Ideally, the injected bone repair material should be administered through the percutaneous or small bone window via precise positioning.

Injectable bone repair materials have gained much attention in recent years due to their numerous potential advantages, such as considerable intensity, steady degradability, minimal damages to tissues and blood supply at the repair site, and great relief of patients' suffering [2–4]. These materials can be used not only for prevention of vertebral fracture, restoration of vertebral height and treatment of osteoporosis, but also for secondary fracture fixation and intramedullary fixation and bone defect and tuberculosis repairs [4–7]. Although injectable bone materials are a relatively new subject, it has become one of the major research focuses in the field of artificial bone repair material research. As the components and structure of natural bone is often the inspiration for bone repair material designs, this review begins with a very brief discussion about the fundamental biology of the natural bone. Then this article summarizes the recent progress made in this field by particularly focusing on the properties and applications of injectable bone materials, including calcium sulfate, calcium phosphate, polymers, calcium phosphate/polymer composites and other materials, to orthopedics and bone tissue repairs, as well as biomimetic fabrication of injectable bone repair materials.

2 The structure and biology of bone

Natural bone matrices are three dimensional organic–inorganic composites with an intricate hierarchical structure [8–10]. The organic materials are composed of 90% of collagen I with 10% of various glycoproteins and proteoglycans. The inorganic material is mainly crystals of hydroxyapatite (HA), which give the bone matrix its stiffness [8]. The hierarchical microstructure of bone matrix includes an orderly deposition of HA minerals within type I collagen matrix. The crystallographic *c*-axis of HA is oriented in parallel to the longitudinal axis of the collagen fibril [11–13]. Figure 1 shows the seven hierarchical levels of organization of natural bone [8].

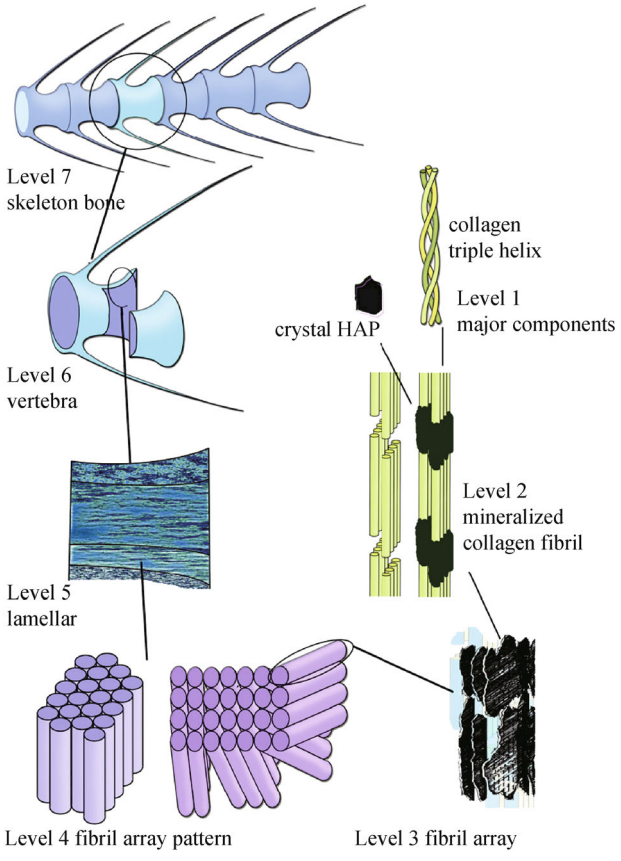


Fig. 1 The seven hierarchical levels of organization of natural zebrafish skeleton bone (Reproduced with permission from Ref. [8]). Level 1: HA crystals and collagen fibrils with the triple helix structure; Level 2: Mineralized collagen fibrils; Level 3: The array of mineralized collagen fibrils; Level 4: Two fibril array patterns of organization found in the bone family of materials; Level 5: The lamellar structure in one vertebra; Level 6: A vertebra; Level 7: Skeleton bone.

As a surrogate of the natural bone, an ideal synthetic injectable bone repair materials for orthopedic and dental application should simulate both the components and the structure of natural bone, moreover, this material should have good biocompatibility, possess suitable working time, suitable rheological properties for injection, exhibit desired mechanical properties, degrade in a controlled manner with resorbable degradation products, and be osteoconductive [14].

3 Current injectable bone repair materials

At present, injectable bone repair materials are mainly divided into the following categories: polymer-based bone materials, calcium phosphate-based bone materials, composite calcium phosphates-polymer-based bone materials, and calcium sulfate-based bone materials. These different categories of bone repair materials will be individually introduced and discussed.

3.1 Polymer-based injectable bone repair materials

Polymeric materials that have been used for bone repairs are usually those, either natural or synthetic, with favorable biocompatibility. Examples of naturally occurring biopolymers used include chitosan, alginic acid and hyaluronic acid, and some of the synthetic polymers are polyesters and polymethylmethacrylate (PMMA).

3.1.1 Injectable natural polymer bone repair materials

Chitosan, alginic acid, and hyaluronic acid (Fig. 2) are natural polysaccharides which are biodegradable and biocompatible. Furthermore, chitosan has multiple free amino groups, whereas alginic acid and hyaluronic acid have multiple carboxyl groups, which makes these polymers easily modified with all kinds of molecular species for the purpose of tuning and improving their physical, chemical and biological properties. Consequently, chitosan, alginic acid, and hyaluronic acid are among the most investigated molecular scaffolds for the development of novel injectable bone repair materials. For example, genipin-crosslinked chitosan has been developed as injectable tissue-engineering scaffold materials and has been used to load live cells [15]. Hydrogels prepared from chitosan derivatives and poly (vinyl alcohol-dimethacrylate-dimethylacrylamide) obtained by photopolymerization have also been used as injectable bone repair materials. Cell culture results showed that this material was good in promoting the cell attachment and proliferation [16]. Alginate has been used not only to encapsulate adipose tissue-derived stromal cells for bone repairs [17] but also to combine with collagen and physiologically active peptides. These composites had improved osteogenic ability [18]. Hydrogels formed by crosslinking hyaluronic acid and polyvinyl alcohol had good osteogenic property and degradability as injectable bone repair material [19]. Moreover, with barium sulfate as radiopaque agent and HA as radiopaque and bioactive agent, hyaluronic acid was combined with PMMA to create injectable low modulus bone cement for the treatment of osteoporosis [20].

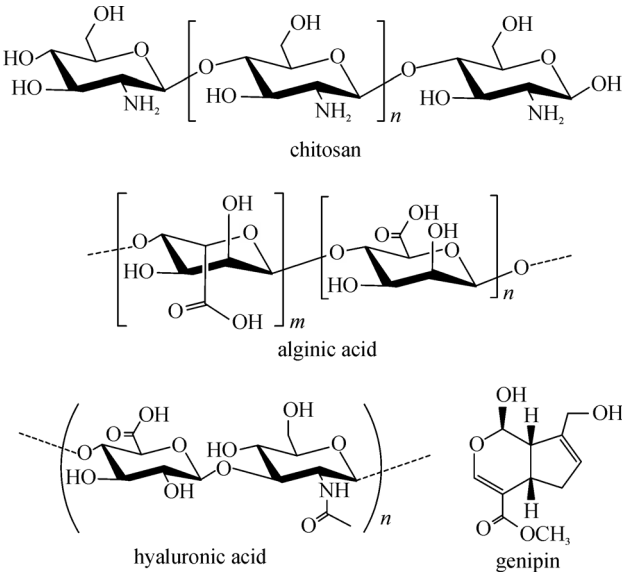


Fig. 2 The structures of chitosan, alginic acid, hyaluronic acid and genipin.

3.1.2 Synthetic polymer-based injectable bone repair materials

Polyesters are a class of polymers derived from the polymerization of organic acids with hydroxyl terminated oligomers. The alternation of different segments in the prepared block co-polymers can provide unique physical, chemical, and biological properties. Many types of polyesters are biocompatible, thus they are good candidates for the development of novel synthetic polymer-based injectable bone repair materials [14,21–24].

Poly(propylene glycol-co-fumaric acid) (PPF) is an unsaturated polyester (Fig. 3(a)) that can be crosslinked in the presence of various fillers and solvent vehicles to yield cements or fillers. PPF filled with calcium gluconate/HA can be fabricated into injectable and absorbable bone materials by crosslinking *in situ*. The materials had injectable viscosity and reasonable working time (6–7 min), hardening time (10–12 min) and osteoconductive property as revealed in the rat femoral osteotomy model [21]. They can also turn into porous materials with good osteo-induction activity when solidified in the sites of damaged tissues [22]. Fluid triblock poly(lactide-block-propylene glycol-block-lactide) dimethacrylate (Fig. 3(b)) was developed as drug-loaded injectable bone repair materials, whose drug release rate could be regulated by adjusting the lengths of lactic acid or propylene glycol

blocks [23]. An *in situ* crosslinkable, biodegradable, methacrylate-encapped porous bone scaffold composed of D- and L-lactide, ε-caprolactone, 1,6-hexanediol and poly(orthoesters) was developed for bone tissue regeneration, which showed excellent biocompatibility and moderate osteoconductive properties, while adding α-TCP could improve its osteoconductive properties. This material also had the potential as a carrier for bone healing promoter substances [24].

Since the degradation products of polycarbonate-based polymers are less acidic than that of the conventionally biodegradable polyesters, such as poly(lactic acid), the former are considered to be more biocompatible. However, most of these polymers are hydrophobic and hence degrade slowly. Incorporating unsaturated groups or hydrophilic segments into polycarbonates can improve its hydrophilicity, degradation rate, mechanical properties, and increase reactive sites for implanting and crosslinking reactions [14]. Due to these desirable properties, a series of injectable, biodegradable, and *in situ* crosslinkable polycarbonate-based polymers have been developed, which include poly(hexamethylene carbonate-fumarate) (PHMCF, Fig. 3(c)), poly(hexamethylene carbonate) diacrylate (PHMCA, Fig. 3(d)), and their amphiphilic copolymers with polyethylene glycol (PEG), poly(ethylene glycol fumarate-co-hexamethylene carbonatefumarate) (PEGF-co-PHMCF, Fig. 3(e)). These polymers have controlled hydrophilicity, biodegradability, and mechanical properties [14].

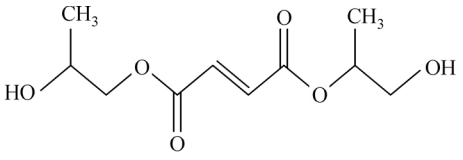
Hydrogels are networks of hydrophilic polymers retaining a large amount of water. In their hydrated form, hydrogels can transport nutrients similar to native tissues, which can be further augmented with biochemical cues to improve the cellular migration and survival after implantation [25]. An example of injectable and biodegradable hydrogels is oligo(poly(ethylene glycol) fumarate), which can be synthesized from fumaryl chloride and PEG. This material can form a hydrogel network by the crosslink of fumarate double bonds in the macromer chains [26]. These hydrogels can encapsulate mesenchymal stem cells (MSCs) for osteochondral repair [27]. However, developing an injectable biomaterial for the encapsulation of viable cells presents a great challenge because of the direct exposure of cells to reactant, products, and so on. To achieve clinical application, additional challenges such as cell viability and storage stability must be considered [25].

Some other polymers with various characteristics have also been developed as injectable bone repair materials, including poly(N-isopropylacrylamide-co-vinylphosphonic acid)-based thermo-responsive hydrogel systems [28], poly(aldehyde guluronate) hydrogels [29], the copolymer of poly(lactic acid) and poly(glycolic acid) (PLA/PGA) [30], polyethylene oxide [31], arginylglycylaspartic acid (RGD)-modified PEG [32] and hydroxypropyl methylcellulose polymer [33].

Although many polymers have been developed as injectable bone repair materials, most of them are still at the research stage. Among these materials, PMMA-based (Fig. 3(f)) implant is one of the most widely studied, and they have also been utilized in clinic owing to their fast setting property and appropriate biocompatibility. Moreover, modifications of PMMA-based bone repair materials have been explored to improve their performance in clinic applications. For example, the fatigue strength of PMMA used for vertebroplasty and kyphoplasty could be adjusted by regulating the solid/liquid ratio of injectable PMMA paste [34]. The property and function of PMMA-based materials could also be improved by incorporating HA containing strontium into PMMA [35]. Putting barium metasilicate and strontium metasilicate into PMMA improved the mechanical property and injectability of these materials [36]. With methyl methacrylate and *N,N*-dimethyltoluidine as the liquid phase, benzoyl peroxide and antibiotics as the solid phase, and bismuth salicylate as the radiopaque agent, injectable crylic acid-based bone cement loaded with antibiotics was developed [37]. Although these modified PMMA materials have improved performances to different extents, there are still limitations in their clinical applications due to the shortcomings of PMMA [38–40]. For example, it has strong exothermic effect (up to 40°C–100°C) when it is solidified, which could easily burn the tissues near the implanted site, especially the nerve roots and the spinal cord. This can also cause burns to the vertebral bone cells to influence the final healing of fracture. PMMA may also release toxic residual monomers that cause hypotension or fat embolism. Moreover, PMMA is lack of bioactivity, bonding ability to the surrounding tissues, osteoconductivity, and biodegradability, and it can result in inflammation as a foreign material at the implanted site. Therefore, many people disapprove treating vertebral diseases by using PMMA, except for the palliative treatment of vertebral metastatic tumors.

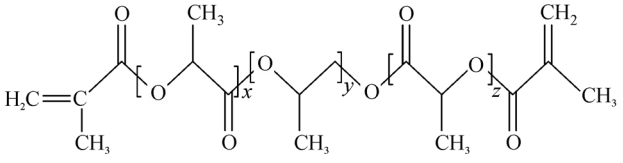
3.2 Calcium phosphate-based injectable bone repair materials

Calcium phosphate has been developed as an *in situ* self-setting bone cement after its hydration and hardening



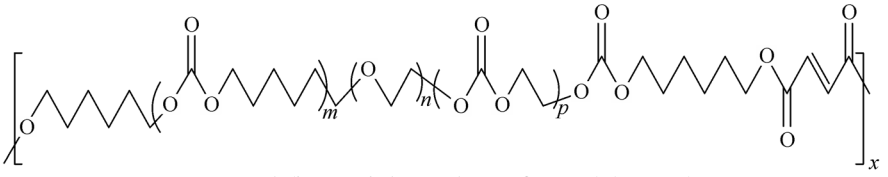
poly(propylene glycol-co-fumaric acid) (PPF)

(a)



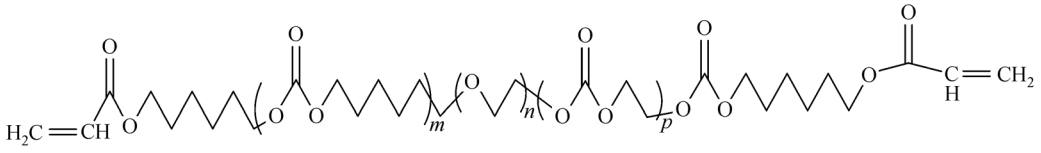
poly(lactide-block-propylene glycol-block-lactide) dimethacrylate

(b)



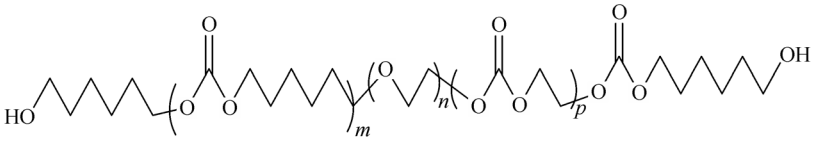
poly(hexamethylene carbonate-fumarate) (PHMCF)

(c)

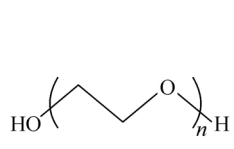


poly(hexamethylene carbonate) diacrylate (PHMCA)

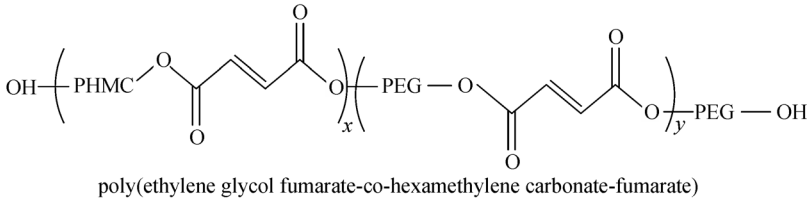
(d)



poly(hexamethylene carbonate) (PHMC)

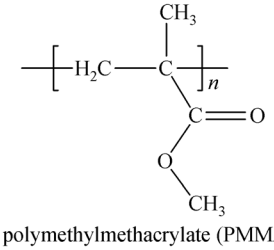


polyethylene glycol (PEG)



poly(ethylene glycol fumarate-co-hexamethylene carbonate-fumarate)

(e)



polymethylmethacrylate (PMMA)

(f)

Fig. 3 The molecular structures of some synthetic polymer-based injectable bone repair materials: **(a)** PPF; **(b)** poly(lactide-block-propylene glycol-block-lactide) dimethacrylate; **(c)** PHMCF; **(d)** PHMCA; **(e)** PHMC, PEG and PEGF-co-PHMCF; **(f)** PMMA.

characteristics were discovered by Chow et al. [41–42]. It can be solidified and shaped freely in the damaged tissue site in the normal environment and under the normal temperature of human body, and eventually converted into HA. Owing to its good bioactivity and biocompatibility, calcium phosphate has satisfactory application to the therapy of bone tissue diseases, such as treatment of lateral tibial plateau fracture [43], enhancement of internal fixation of femoral neck fracture [44], joint internal fixation [45], maxillary repair [46], alveolar ridge augmentation [47], bone defect repair, and so on. It can also be used as a drug carrier. For example, controlled release of cephalosporin antibiotics was achieved by mixing the antibiotics with the calcium phosphate cement and adjusting the cement pore size, and neither the percent conversion of calcium phosphate to apatite phase nor the drug activity was affected [48].

Due to the many advantages of calcium phosphate as bone cement, such as free shape, *in situ* self-setting property, good biocompatibility and gradual degradability, it has the potential to be developed into a series of new injectable bone repair materials. Because biocompatible HA as one of the main components of natural bones can effectively promote osteoinductive activity, the application of injectable calcium phosphate bone cements transformable into HA can lead to revolutionary therapies for bone defects. Nonetheless, the calcium phosphate bone cement does have defects such as easy collapsibility in body fluids and poor degradability and mechanical properties, so it is necessary to improve these properties of the new injectable calcium phosphate bone cements in order to make them applicable in clinic.

At present, there are many reports on using calcium phosphate as injectable bone cement. Its injectability can be improved by adjusting the rheological behavior of concentrated calcium phosphate cement slurry [49]. The pore-forming property of calcium phosphate cement could be enhanced by using acetic acid and citric acid as pore-making agents [50]. The setting time of calcium hydrogen phosphate was shortened upon adding sodium phosphate as a setting accelerator, and the refrangibility and mechanical property of calcium hydrogen phosphate were significantly improved by adding PMMA [30,51]. The HA and β-calcium phosphate complex was developed as an injectable, bone marrow-containing, and two-phase bone repair material with improved osteoconductive property [52]. *In vivo* histological studies have also demonstrated that nanocrystal HA has better osteogenic property and vascular regeneration performance [53].

It has been found that the bone repair ability of the calcium phosphate cement could also be improved by adding other elements or components. For example, adding calcium carbonate could improve the osteogenic property [54], and the addition of calcium silicate could promote material mineralization and cell proliferation on the material [55]. Calcium phosphate loaded with zinc element could maintain zinc release for a long period, which can increase the bone mineralization density and be especially useful in the treatment of zinc-deficient bone defects [56]. Calcium phosphate loaded with magnesium, zinc and fluoride elements had increased bone mineralization density and mechanical strength [57]. Adding magnesium phosphate not only improved the injectability, setting time, and mechanical property of materials, but also modified their degradability [58]. The addition of hexadecyl trimethyl ammonium bromide led to macroporous injectable bone repair materials [59], which made it easy for bone tissues to grow into it and to integrate with the material [60]. The calcium phosphate cement containing strontium carbonate had better injectability and compressive strength, which had an effect on the distribution of pores in the cement [61], whereas bioactive HA bone cement containing strontium element with processed surface by methyl methacrylate had improved mechanical properties [62]. The mixture of calcium phosphate and calcium sulfate had not only good injectability, appropriate setting time and mechanical property but also improved degradability and osteogenetic ability [63].

3.3 Calcium phosphate-polymer composites as injectable bone repair materials

Natural bone matrices are complex tissues, which are composed of organic matrices with deposited HA crystals. Introducing polymer materials into calcium phosphate can mimic natural bones and improve the performance during bone repair. Combining calcium phosphate or biphasic calcium phosphate with polycaprolactone (PCL) [64], acrylic acid [65] or hydroxypropyl methyl cellulose [66–67] could improve their mechanical and other properties. Compared to macroporous biphasic calcium phosphate, the composites of hydroxypropyl methyl cellulose and calcium phosphate as injectable bone materials had improved biological activity at the early implantation stage. The material micropores between the crystal particles of biphasic calcium phosphate and hydroxypropyl methyl cellulose could help the permeation of body fluids into the materials and promoted angiogenesis and three-dimen-

sional cell migration, growth, and new bone generation [68]. The poly(propylene fumarate) and calcium phosphate composites as injectable bone materials can be used for the treatment of femoral head necrosis, although poly(propylene fumarate) has some toxicity. Both the biocompatibility and the mechanical property of the composites were enhanced with the increase of calcium phosphate content in the composites [69–70].

The calcium phosphate and natural polymer complexes, such as the composites of HA and collagen prepared by the sol-gel method [71] or by collagen coating or HA capsules [72] had additional advantages as injectable bone repair materials due to the inherent biocompatibility of natural polymers. Introduction of calcium alginate into the HA-collagen composites could modify their injectability and setting times [73]. The composites of β-calcium phosphate and collagen loaded with fluvastatin were used for vertical bone augmentation [74]. The addition of recombinant human platelet-derived growth factor into the collagen-β tricalcium phosphate composites could accelerate fracture repair [75]. The calcium phosphate-gelatin composites had good osteogenic properties and enhanced the mechanical strength of new bones, while bone remodeling was improved with the addition of transforming growth factor-β1 (TGF-β1) [76]. Adding hyaluronic acid into calcium phosphate not only shortened the setting time but also improved the physicochemical and biological properties, such as the anti-collapsibility, mechanical property, degradability and bioactivity [77–78]. Compared to calcium phosphate approved by U.S. Food and Drug Administration (FDA), the calcium phosphate and chitosan composites had comparative or better mechanical properties, biocompatibility and osteogenic properties [79]. The β-calcium phosphate particle-fibrin-thrombin gel composites are another class of promising injectable bone repair materials, in which calcium phosphate particles are distribute in fibrin gel. The elastic modulus and bioactivity of these materials were improved with the increase of calcium phosphate content in the composites [80].

In short, the synergistic effect of calcium phosphate and polymers can help overcome their respective limitations as bone repair materials. Consequently, the calcium phosphate-polymer composites are becoming one of the most promising research directions.

3.4 Calcium sulfate-based injectable bone repair materials

Calcium sulfate is considered as one of the most promising bone graft substitute materials because of its good biocompatibility, degradability and no irritation to the surrounding tissues, thus it has been studied and used for filling bone defects for a long time [81–82]. The calcium sulfate used in clinic is α-calcium sulfate hemihydrate (CSH) that has intact crystal structure and good plasticity and *in situ* self-setting properties. In particular, when mixed with water, CSH is converted into calcium sulfate dihydrate (CSD) with high density and strength. In the course of conversion, the material can be shaped into different forms, which are very suitable for filling or repairing bone defects. At present, calcium sulfate has been approved by both FDA and Conformite Europeenne (CE) as injectable bone materials in wide clinic use. In the treatment of osteoporosis, calcium sulfate can enhance the fixation of internal bone nails, which reduces the risk of graft failure [83]. In the treatment of aneurysmal bone cyst, calcium sulfate can promote new bone formation with its own degradation [84]. It also has good effect in the treatment of tibial plateau fracture [85].

Although calcium sulfate has many advantages as bone repair materials, it also has some problems, such as too rapid degradation and lack of osteoinductive activity. Whereas future studies should address these problems so as to match the degradation rate of calcium sulfate with the new bone formation rate and to improve its osteoinductivity, newly designed calcium sulfate-based bone materials should also have improved operability, injectability, mechanical properties and biocompatibility, for minimally invasive treatment of bone trauma.

At present, many efforts have been devoted to improve the properties of calcium sulfate by adding other elements or components. When calcium sulfate and calcium phosphate are mixed together, the resulting materials have suitable injectability and mechanical properties, adjustable setting time, and good ability to promote new bone formation [58]. Modifying the injectable calcium phosphate-calcium sulfate composite with iron ion could further improve the injectability and mechanical properties, and the resulting materials could be used to treat spinal diseases caused by osteoporosis [86]. The properties of calcium sulfate may also be improved by combining with polymers. The poly(lactic-co-glycolic acid) (PLGA)-polyvinyl alcohol (PVA)-calcium sulfate-cellulose ether composites were developed as injectable carriers for the delivery of growth factors in the treatment of orthopedic and periodontal diseases [87]. Injectable bone materials prepared by using citric acid and chitosan as the liquid

phase and calcium phosphate and calcium sulfate hemihydrate as the solid phase had much improved injectability, crystallinity and mechanical properties [88].

4 Biomimetic injectable bone repair materials

An ideal bone repair material should resemble natural bones in both the composition and the microstructure. To achieve such materials, much effort has been attempted by employing the biomimetic strategy *in vitro*. Biomimetic bone repair materials with certain 3-dimensional (3D) structures can be prepared via self-assembly of proper materials, such as assembly of biomimetic mineralized collagen from collagen triple helices and nano-HA [89]. The crystallographic *c*-axis of nano-HA is oriented in parallel to each other and to the longitudinal axis of collagen molecules and fibrils in which they are located. The deposited collagen fibrils then align in parallel to each other, assembling into mineralized collagen fibers, which have similar hierarchical microstructure and main chemical components as those of natural bones. This has been verified by both conventional and high-resolution transmission electron microscopy (HRTEM) (Fig. 4) [89]. Figure 5 shows the schematic depiction of the self-assembled nano-HA/collagen composites comprising hierarchical microstructure. The lowest level of this hierarchical structure is the organization of HA crystals and collagen fibrils with the triple helix structure of molecule. The collagen fibrils are formed by self-assembly of the triple helix structure of collagen molecule, and the HA crystals are deposited initially in the gap zones between the collagen fibrils. The second level of the hierarchical structure is the formation of the collagen fibrils with HA crystals growth on these fibrils. The *c*-axes of HA crystals are oriented along the longitudinal axes of collagen molecule and the fibrils. This organization implies that the nucleation and growth of HA crystals on these fibrils are controlled by the fibrils. The third level of the hierarchical structure is the organization of the mineralized collagen fibrils that are aligned in parallel to each other to form mineralized collagen fiber bundles [8,89].

Studies have shown that biomimetic mineralized collagen is an excellent bone repair material with good biocompatibility and osteoinductivity [8,90], and it has already been successfully utilized in tens of thousands of cases in clinic, including different kinds of hard tissue repairs, such as bone defect, lumbar and neck hurt, and so

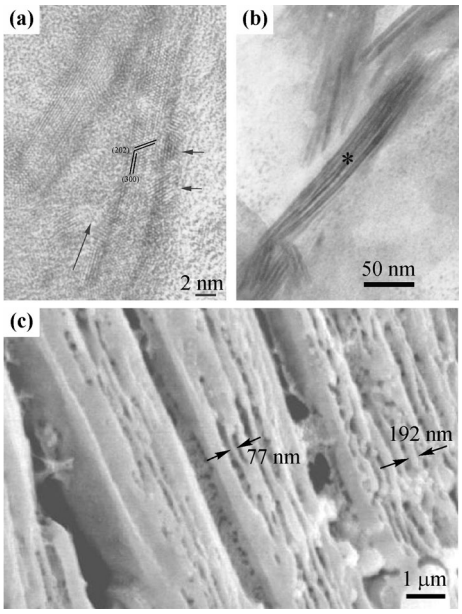


Fig. 4 Hierarchical structure of biomimetic mineralized collagen: **(a)** long arrow indicates the longitude direction of collagen fibril, and two short arrows indicate HA nanocrystals; **(b)** mineralized collagen fibrils; **(c)** mineralized collagen fibers. (Reproduced with permission from Ref. [89])

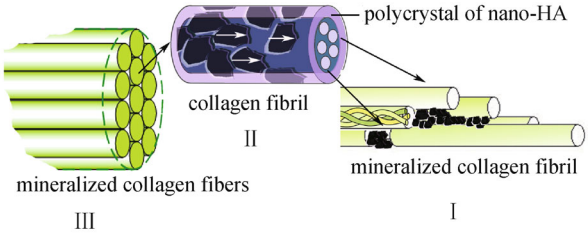


Fig. 5 Scheme of the hierarchical structure of biomimetic mineralized collagen. (Reproduced with permission from Ref. [8])

on. In all these cases, the wounds heal and no abnormity has been found with local and systematic examinations during long-term follow-up [8]. However, the present implants of bone repair material lack handling properties due to its solid-preformed block form and a lack of the desired shapes. Surgeons have to match the surgical sites with the implants, which can lead to increased bone trauma to surrounding tissues and longer surgery time. To overcome the problems, CSH as a setting agent was introduced into the mineralized collagen to explore an injectable bone repair material with good injectability and self-setting property [91]. The resulted composites possessed good biocompatibility, mechanical property, and osteoinductivity [91–93]. Cells interacted with them well, and moreover, multiple layers of cells could be formed on

the material (Fig. 6). These results imply that the biomimetic materials favor cell adhesion and proliferation. Figure 7 shows the histological hematoxylin and eosin (HE) staining results of this material implantation into a bone defect. It is clear that the bone defect was gradually repaired 12 weeks later. This means that the material could provide a suitable biological environment for new bone growth at the implant site. By combining with minimally invasive surgical techniques, the material can be developed into an excellent injectable bone repair material, which will not only mold to the shape of the tissue cavity by setting *in situ* after injection, but also decrease the surgery time, reduce trauma, decrease the size of the scars and relieve the suffering of patients [91]. The patient can also achieve rapid recovery in a cost-effective manner, so to greatly improve the patient's quality of life. Therefore, biomimetic materials are one of the most candidates for injectable bone repair materials.

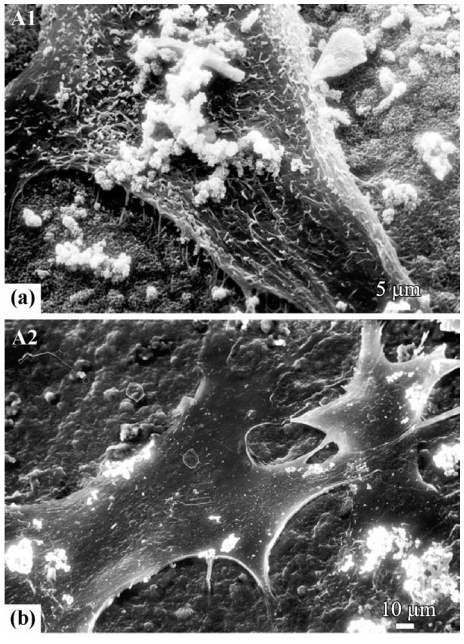


Fig. 6 SEM results of 2-day cultured marrow stromal cells on injectable bone repair materials: **(a)** interaction between cell and materials; **(b)** interactions between cell and cell on the materials. (Reproduced with permission from Ref. [91])

5 Summary and outlook

The field of injectable bone repair material development has witnessed great progress in recent years. Despite that, many of the materials still have problems that restrict their clinical applications. For instance, although PMMA and other synthetic polymer-based materials have already been utilized clinically, their strong exothermic effect upon solidification can burn the surrounding tissues and affect bone healing. Moreover, many of these materials can release toxic residual monomers that may cause hypotension or embolism, or lack osteoconduction, biodegradability, and osseointegration properties, forcing them to exist in the body as foreign materials that can cause inflammatory responses. Some of the problems of calcium phosphate as a bone repair material are its easy collapsibility in body fluids, poor mechanical properties, low porosity, and slow degradability. Potential solutions to overcome these shortcomings of calcium phosphate are to modulate its properties via adding other components or to develop nano-HA and nano-HA-based organic-inorganic hybrid materials. The use of nano-HA may improve their degradability and their binding to surrounding tissues. The synergistic effect of organic and inorganic materials may enhance the anti-collapsibility, mechanical property, and porosity of the resulting hybrid materials. The drawbacks of calcium sulfate as a bone repair material are its relatively rapid degradation, easy collapsibility in body fluids, and the lack of osteoinductivity. Future research should focus on how to improve its degradation, mechanical, and osteoinductive properties, and in the meantime its injectability [94]. Clearly, a universally useful strategy to overcome the shortcomings of individual materials is composite materials, which have shown promising properties in many cases. While composite materials can be realized by combining inorganic materials and polymers, including both natural and synthetic polymers, and other components, the physical, chemical, mechanical and biological properties of the resulting composites can be further modified and improved through careful design and revision of the material composition and other parameters.

Despite some advantages of the manmade bone repair materials, the best bone implant material used in clinic is still autologous bone, which has widely been accepted as the gold standard for the treatment of bone defects. Therefore, biomimetic bone materials mimicking both the composition and the 3D structure of natural bones have great promises and are the potentially fruitful direction of injectable bone repair materials. These materials should be adequately integrated with the encoded spatial and temporal cues of the extracellular matrix (ECM), which will possess proper physical, chemical, mechanical and biological properties similar to that of the host microenvironment so as to promote cell attachment, proliferation, and bone repair or regeneration.

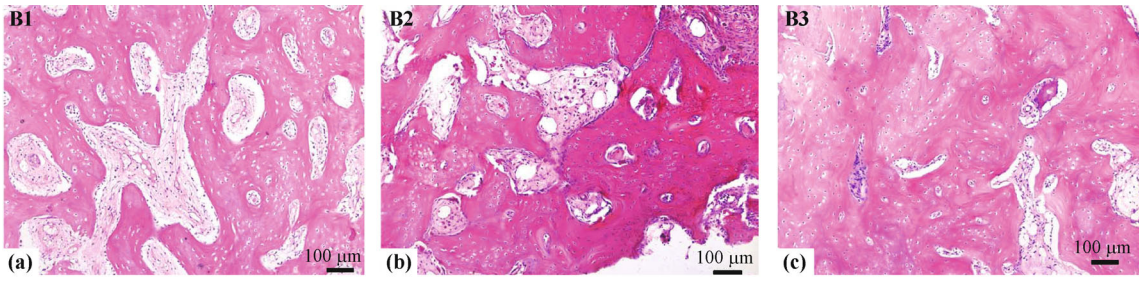


Fig. 7 Histological HE staining results of implanted injectable bone repair materials: **(a)** implant at 4 weeks; **(b)** implant at 8 weeks; **(c)** implant at 12 weeks. (Reproduced with permission from Ref. [91])

Bone defect-related infections such as osteomyelitis are quite common in clinic, which seriously affect osseointegration and are usually regarded as contraindications for bone implant. Conventional treatments such as surgical debridement and suction irrigation can only control but not treat local infections [95]. Therefore, local delivery of antibiotics is desirable in treating osteomyelitis or preventing contaminations. A number of studies have reported using bone substitutes to deliver antibiotics locally for open fractures, infection prevention, and osteomyelitis [96]. These bone substitutes include calcium sulfate, calcium phosphate and biodegradable polymers, synthetic or natural, such as PLA, PGA, collagen and chitosan [97–98]. Therefore, antibiotic-loaded injectable bone repair materials are also becoming more and more important [98–100].

In brief, injectable bone repair materials have already shown great promises and successes in clinical applications owing to the advantage of little tissue damage, easy handling, and few complications after surgery. All the same, there are some shortcomings to restrict the further development and applications of injectable bone repair material, especially the urgent issues of improving bone repair capacity and the cytocompatibility of materials.

Abbreviations

CSD	calcium sulfate dihydrate
CSH	α-calcium sulfate hemihydrate
ECM	extracellular matrix
HA	hydroxyapatite
HE	hematoxylin and eosin
HRTEM	high-resolution transmission electron microscopy
MSC	mesenchymal stem cell
PCL	polycaprolactone
PEG	polyethylene glycol
PEGF	poly(ethylene glycol fumarate)
PGA	poly(glycolic acid)
PHMCA	poly(hexamethylene carbonate) diacrylate
PHMCF	poly(hexamethylene carbonate-fumarate)
PLA	poly(lactic acid)
PLGA	poly(lactic-co-glycolic acid)
PMMA	polymethylmethacrylate
PPF	poly(propylene glycol-co-fumaric acid)
PVA	polyvinyl alcohol
RGD	arginylglycylaspartic acid
SEM	scanning electron microscopy
TGF	transforming growth factor

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References

[1] Hench L L, Polak J M. Third-generation biomedical materials. Science, 2002, 295(5557): 1014–1017

[2] Dreifke M B, Ebraheim N A, Jayasuriya A C. Investigation of potential injectable polymeric biomaterials for bone regeneration. Journal of Biomedical Materials Research Part A, 2013, 101 (8): 2436–2447

[3] He Y, Gao J, Li X, et al. Fabrication of injectable calcium sulfate bone graft material. Journal of Biomaterials Science: Polymer Edition, 2010, 21(10): 1313–1330

[4] Low K L, Tan S H, Zein S H S, et al. Calcium phosphate-based composites as injectable bone substitute materials. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2010, 94(1): 273–286

[5] Hile D D, Kowaleski M P, Doherty S A, et al. An injectable porous poly(propylene glycol-co-fumaric acid) bone repair material as an adjunct for intramedullary fixation. Bio-Medical

Materials and Engineering, 2005, 15(3): 219–227

[6] Yang X, Gan Y, Gao X, et al. Preparation and characterization of trace elements-multidoped injectable biomimetic materials for minimally invasive treatment of osteoporotic bone trauma. Journal of Biomedical Materials Research Part A, 2010, 95(4): 1170–1181

[7] Zhu X S, Zhang Z M, Mao H Q, et al. A novel sheep vertebral bone defect model for injectable bioactive vertebral augmentation materials. Journal of Materials Science: Materials in Medicine, 2011, 22(1): 159–164

[8] Cui F Z, Li Y, Ge J. Self-assembly of mineralized collagen composites. Materials Science and Engineering R: Reports, 2007, 57(1–6): 1–27

[9] Wang X M, Cui F Z, Ge J, et al. Hierarchical structural comparisons of bones from wild-type and liliput(dtc232) gene-mutated Zebrafish. Journal of Structural Biology, 2004, 145(3): 236–245

[10] Weiner S, Wagner H D. The material bone: Structure mechanical function relations. Annual Review of Materials Science, 1998, 28 (1): 271–298

[11] Cui F Z, Wen H B, Su X W, et al. Microstructures of external periosteal callus of repaired femoral fracture in children. Journal of Structural Biology, 1996, 117(3): 204–208

[12] Landis W J, Song M J, Leith A, et al. Mineral and organic matrix interaction in normally calcifying tendon visualized in three dimensions by high-voltage electron microscopic tomography and graphic image reconstruction. Journal of Structural Biology, 1993, 110(1): 39–54

[13] Weiner S, Traub W. Organization of hydroxyapatite crystals within collagen fibrils. FEBS Letters, 1986, 206(2): 262–266

[14] Sharifi S, Imani M, Mirzadeh H, et al. Synthesis, characterization, and biocompatibility of novel injectable, biodegradable, and *in situ* crosslinkable polycarbonate-based macromers. Journal of Biomedical Materials Research Part A, 2009, 90(3): 830–843

[15] Cruz D M, Ivirico J L, Gomes M M, et al. Chitosan microparticles as injectable scaffolds for tissue engineering. Journal of Tissue Engineering and Regenerative Medicine, 2008, 2(6): 378–380

[16] Ma G, Yang D, Li Q, et al. Injectable hydrogels based on chitosan derivative/polyethylene glycol dimethacrylate/N,N-dimethylacrylamide as bone tissue engineering matrix. Carbohydrate Polymers, 2010, 79(3): 620–627

[17] Abbah S A, Lu W W, Chan D, et al. *In vitro* evaluation of alginate encapsulated adipose-tissue stromal cells for use as injectable bone graft substitute. Biochemical and Biophysical Research Communications, 2006, 347(1): 185–191

[18] Lee J Y, Choo J E, Park H J, et al. Injectable gel with synthetic collagen-binding peptide for enhanced osteogenesis *in vitro* and *in vivo*. Biochemical and Biophysical Research Communications, 2007, 357(1): 68–74

[19] Bergman K, Engstrand T, Hilborn J, et al. Injectable cell-free template for bone-tissue formation. Journal of Biomedical Materials Research Part A, 2009, 91(4): 1111–1118

[20] Boger A, Bohner M, Heini P, et al. Properties of an injectable low modulus PMMA bone cement for osteoporotic bone. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2008, 86(2): 474–482

[21] Lewandrowski K U, Gresser J D, Wise D L, et al. Osteoconductivity of an injectable and bioresorbable poly(propylene glycol-co-fumaric acid) bone cement. Biomaterials, 2000, 21(3): 293–298

[22] Kim C W, Talac R, Lu L, et al. Characterization of porous injectable poly-(propylene fumarate)-based bone graft substitute. Journal of Biomedical Materials Research Part A, 2008, 85(4): 1114–1119

[23] Young A M, Ho S M. Drug release from injectable biodegradable polymeric adhesives for bone repair. Journal of Controlled Release, 2008, 127(2): 162–172

[24] Vertenten G, Vlaminck L, Gorski T, et al. Evaluation of an injectable, photopolymerizable three-dimensional scaffold based on D,L-lactide and ε-caprolactone in a tibial goat model. Journal of Materials Science: Materials in Medicine, 2008, 19(7): 2761–2769

[25] Page J M, Harmata A J, Guelcher S A. Design and development of reactive injectable and settable polymeric biomaterials. Journal of Biomedical Materials Research Part A, 2013, 101 (12): 3630–3645

[26] Shin H, Quinten Ruhé P, Mikos A G, et al. *In vivo* bone and soft tissue response to injectable, biodegradable oligo(poly(ethylene glycol) fumarate) hydrogels. Biomaterials, 2003, 24(19): 3201–3211

[27] Guo X, Park H, Liu G, et al. *In vitro* generation of an osteochondral construct using injectable hydrogel composites encapsulating rabbit marrow mesenchymal stem cells. Biomaterials, 2009, 30(14): 2741–2752

[28] Kim S Y, Lee S C. Thermo-responsive injectable hydrogel system based on poly(N-isopropylacrylamide-co-vinylphosphonic acid). I. Biomineralization and protein delivery. Journal of Applied Polymer Science, 2009, 113(6): 3460–3469

[29] Lee K Y, Alsberg E, Mooney D J. Degradable and injectable poly (aldehyde guluronate) hydrogels for bone tissue engineering. Journal of Biomedical Materials Research, 2001, 56(2): 228–233

[30] Rimondini L, Nicoli-Aldini N, Fini M, et al. *In vivo* experimental study on bone regeneration in critical bone defects using an injectable biodegradable PLA/PGA copolymer. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodon-

tics, 2005, 99(2): 148–154

[31] Chen F, Mao T, Tao K, et al. Injectable bone. The British Journal of Oral & Maxillofacial Surgery, 2003, 41(4): 240–243

[32] Burdick J A, Anseth K S. Photoencapsulation of osteoblasts in injectable RGD-modified PEG hydrogels for bone tissue engineering. Biomaterials, 2002, 23(22): 4315–4323

[33] Amouriq Y, Bourges X, Weiss P, et al. Skin sensitization study of two hydroxypropyl methylcellulose components (Benecel and E4M) of an injectable bone substitute in guinea pigs. Journal of Materials Science: Materials in Medicine, 2002, 13(2): 149–154

[34] Lewis G, Koole L H, van Hooy-Corstjens C S J. Influence of powder-to-liquid monomer ratio on properties of an injectable iodine-containing acrylic bone cement for vertebroplasty and balloon kyphoplasty. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2009, 91(2): 537–544

[35] Hernández L, Parra J, Vázquez B, et al. Injectable acrylic bone cements for vertebroplasty based on a radiopaque hydroxyapatite. Bioactivity and biocompatibility. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2009, 88(1): 103–114

[36] Carrodeguas R G, Lasa B V, Del Barrio J S R. Injectable acrylic bone cements for vertebroplasty with improved properties. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2004, 68(1): 94–104

[37] Hernandez L, Muñoz M E, Goñi I, et al. New injectable and radiopaque antibiotic loaded acrylic bone cements. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2008, 87(2): 312–320

[38] Webb J C J, Spencer R F. The role of polymethylmethacrylate bone cement in modern orthopaedic surgery. Journal of Bone and Joint Surgery (British Volume), 2007, 89(7): 851–857

[39] Robinson Y, Tschöke S, Stahel P F, et al. Complications and safety aspects of kyphoplasty for osteoporotic vertebral fractures: a prospective follow-up study in 102 consecutive patients. Patient Safety in Surgery, 2008, 2(1): 2 (10 pages)

[40] Kalteis T, Lüring C, Gugler G, et al. Acute tissue toxicity of PMMA bone cements. Zeitschrift für Orthopädie und ihre Grenzgebiete, 2004, 142(6): 666–672

[41] Brown W E, Chow L C. A new calcium phosphate setting cement. Journal of Dental Research, 1983, 62(1): 672–679

[42] Gruninger S E S C, Chow L C, O'young A, et al. Evaluation of the biocompatibility of a new calcium phosphate setting cement. Journal of Dental Research, 1984, 63: 200

[43] Horstmann W G, Verheyen C C, Leemans R. An injectable calcium phosphate cement as a bone-graft substitute in the treatment of displaced lateral tibial plateau fractures. Injury, 2003, 34(2): 141–144

[44] Stankewich C J, Swiontkowski M F, Tencer A F, et al. Augmentation of femoral neck fracture fixation with an injectable calcium–phosphate bone mineral cement. Journal of Orthopaedic Research, 1996, 14(5): 786–793

[45] Zimmermann R, Gabl M, Lutz M, et al. Injectable calcium phosphate bone cement Norian SRS for the treatment of intra-articular compression fractures of the distal radius in osteoporotic women. Archives of Orthopaedic and Trauma Surgery, 2003, 123 (1): 22–27

[46] Aral A, Yalçin S, Karabuda Z C, et al. Injectable calcium phosphate cement as a graft material for maxillary sinus augmentation: an experimental pilot study. Clinical Oral Implants Research, 2008, 19(6): 612–617

[47] Sato I, Akizuki T, Oda S, et al. Histological evaluation of alveolar ridge augmentation using injectable calcium phosphate bone cement in dogs. Journal of Oral Rehabilitation, 2009, 36 (10): 762–769

[48] Hesaraki S, Nemati R. Cephalexin-loaded injectable macroporous calcium phosphate bone cement. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2009, 89(2): 342–352

[49] Liu C, Shao H, Chen F, et al. Rheological properties of concentrated aqueous injectable calcium phosphate cement slurry. Biomaterials, 2006, 27(29): 5003–5013

[50] Hesaraki S, Zamanian A, Moztarzadeh F. The influence of the acidic component of the gas-foaming porogen used in preparing an injectable porous calcium phosphate cement on its properties: acetic acid versus citric acid. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2008, 86(1): 208–216

[51] Ryf C, Goldhahn S, Radziejowski M, et al. A new injectable brushite cement: first results in distal radius and proximal tibia fractures. European Journal of Trauma and Emergency Surgery, 2009, 35(4): 389–396

[52] Lerouxel E, Weiss P, Giumelli B, et al. Injectable calcium phosphate scaffold and bone marrow graft for bone reconstruction in irradiated areas: an experimental study in rats. Biomaterials, 2006, 27(26): 4566–4572

[53] Laschke M W, Witt K, Pohlemann T, et al. Injectable nanocrystalline hydroxyapatite paste for bone substitution: *in vivo* analysis of biocompatibility and vascularization. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2007, 82(2): 494–505

[54] Wolff K D, Swaid S, Nolte D, et al. Degradable injectable bone cement in maxillofacial surgery: indications and clinical experience in 27 patients. Journal of Cranio-Maxillo-Facial Surgery, 2004, 32(2): 71–79

[55] Li J, Qiu Z Y, Zhou L, et al. Novel calcium silicate/calcium phosphate composites for potential applications as injectable bone cements. Biomedical Materials, 2008, 3(4): 044102

[56] Otsuka M, Ohshita Y, Marunaka S, et al. Effect of controlled zinc release on bone mineral density from injectable Zn-containing β-tricalcium phosphate suspension in zinc-deficient diseased rats. Journal of Biomedical Materials Research Part A, 2004, 69(3): 552–560

[57] Otsuka M, Oshinbe A, Legeros R Z, et al. Efficacy of the injectable calcium phosphate ceramics suspensions containing magnesium, zinc and fluoride on the bone mineral deficiency in ovariectomized rats. Journal of Pharmaceutical Sciences, 2008, 97(1): 421–432

[58] Wu F, Su J, Wei J, et al. Injectable bioactive calcium–magnesium phosphate cement for bone regeneration. Biomedical Materials, 2008, 3(4): 1873–1884

[59] Wang X, Ye J, Li X, et al. Production of *in-situ* macropores in an injectable calcium phosphate cement by introduction of cetyl-trimethyl ammonium bromide. Journal of Materials Science: Materials in Medicine, 2008, 19(10): 3221–3225

[60] del Valle S, Miño N, Muñoz F, et al. *In vivo* evaluation of an injectable macroporous calcium phosphate cement. Journal of Materials Science: Materials in Medicine, 2007, 18(2): 353–361

[61] Wang X, Ye J, Wang Y. Influence of a novel radiopacifier on the properties of an injectable calcium phosphate cement. Acta Biomaterialia, 2007, 3(5): 757–763

[62] Zhao F, Lu W W, Luk K D K, et al. Surface treatment of injectable strontium-containing bioactive bone cement for vertebroplasty. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2004, 69(1): 79–86

[63] Hu G, Xiao L, Fu H, et al. Study on injectable and degradable cement of calcium sulphate and calcium phosphate for bone repair. Journal of Materials Science: Materials in Medicine, 2010, 21(2): 627–634

[64] Iooss P, Le Ray A M, Grimandi G, et al. A new injectable bone substitute combining poly(ε-caprolactone) microparticles with biphasic calcium phosphate granules. Biomaterials, 2001, 22 (20): 2785–2794

[65] Rodríguez-Lorenzo L M, Fernández M, Parra J, et al. Acrylic injectable and self-curing formulations for the local release of bisphosphonates in bone tissue. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2007, 83(2): 596–608

[66] Blouin S, Moreau M F, Weiss P, et al. Evaluation of an injectable bone substitute (βTCP/hydroxyapatite/hydroxy-propyl-methyl-cellulose) in severely osteopenic and aged rats. Journal of Biomedical Materials Research Part A, 2006, 78(3): 570–580

[67] Weiss P, Layrolle P, Clergeau L P, et al. The safety and efficacy of an injectable bone substitute in dental sockets demonstrated in a human clinical trial. Biomaterials, 2007, 28(22): 3295–3305

[68] Gauthier O, Goyenvalle E, Bouler J M, et al. Macroporous biphasic calcium phosphate ceramics versus injectable bone substitute: a comparative study 3 and 8 weeks after implantation in rabbit bone. Journal of Materials Science: Materials in Medicine, 2001, 12(5): 385–390

[69] Chang C H, Liao T C, Hsu Y M, et al. A poly(propylene fumarate)–calcium phosphate based angiogenic injectable bone cement for femoral head osteonecrosis. Biomaterials, 2010, 31 (14): 4048–4055

[70] Peter S J, Kim P, Yasko A W, et al. Crosslinking characteristics of an injectable poly(propylene fumarate)/β-tricalcium phosphate paste and mechanical properties of the crosslinked composite for use as a biodegradable bone cement. Journal of Biomedical Materials Research, 1999, 44(3): 314–321

[71] Habraken W J E M, de Jonge L T, Wolke J G C, et al. Introduction of gelatin microspheres into an injectable calcium phosphate cement. Journal of Biomedical Materials Research Part A, 2008, 87(3): 643–655

[72] Link D P, van den Dolder J, van den Beucken J J, et al. Bone response and mechanical strength of rabbit femoral defects filled with injectable CaP cements containing TGF-β1 loaded gelatin microparticles. Biomaterials, 2008, 29(6): 675–682

[73] Kai D, Li D, Zhu X, et al. Addition of sodium hyaluronate and the effect on performance of the injectable calcium phosphate cement. Journal of Materials Science: Materials in Medicine, 2009, 20(8): 1595–1602

[74] Chazono M, Tanaka T, Komaki H, et al. Bone formation and bioresorption after implantation of injectable β-tricalcium phosphate granules-hyaluronate complex in rabbit bone defects. Journal of Biomedical Materials Research Part A, 2004, 70(4): 542–549

[75] Pek Y S, Kurisawa M, Gao S, et al. The development of a nanocrystalline apatite reinforced crosslinked hyaluronic acid-tyramine composite as an injectable bone cement. Biomaterials, 2009, 30(5): 822–828

[76] Plachokova A, Link D, van den Dolder J, et al. Bone regenerative properties of injectable PGLA–CaP composite with TGF-β1 in a rat augmentation model. Journal of Tissue Engineering and Regenerative Medicine, 2007, 1(6): 457–464

[77] Moreau J L, Xu H H K. Mesenchymal stem cell proliferation and differentiation on an injectable calcium phosphate–chitosan composite scaffold. Biomaterials, 2009, 30(14): 2675–2682

[78] Liu H, Li H, Cheng W, et al. Novel injectable calcium phosphate/chitosan composites for bone substitute materials. Acta Biomaterialia, 2006, 2(5): 557–565

[79] Montufar E B, Traykova T, Gil C, et al. Foamed surfactant solution as a template for self-setting injectable hydroxyapatite scaffolds for bone regeneration. Acta Biomaterialia, 2010, 6(3): 876–885

[80] Jayabalan M, Shalumon K T, Mitha M K. Injectable biomaterials

for minimally invasive orthopedic treatments. Journal of Materials Science: Materials in Medicine, 2009, 20(6): 1379–1387

[81] Yang G J, Lin M, Zhang L, et al. Progress of calcium sulfate and inorganic composites for bone defect repair. Journal of Inorganic Materials, 2013, 28(8): 795–803

[82] Peltier L F, Bickel E Y, Lillo R, et al. The use of plaster of paris to fill defects in bone. Annals of Surgery, 1957, 146(1): 61–69

[83] Yu X W, Xie X H, Yu Z F, et al. Augmentation of screw fixation with injectable calcium sulfate bone cement in ovariectomized rats. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2009, 89(1): 36–44

[84] Clayer M. Injectable form of calcium sulphate as treatment of aneurysmal bone cysts. ANZ Journal of Surgery, 2008, 78(5): 366–370

[85] Yu B, Han K, Ma H, et al. Treatment of tibial plateau fractures with high strength injectable calcium sulphate. International Orthopaedics, 2009, 33(4): 1127–1133

[86] Vlad M D, del Valle L J, Poeata I, et al. Injectable iron-modified apatitic bone cement intended for kyphoplasty: cytocompatibility study. Journal of Materials Science: Materials in Medicine, 2008, 19(12): 3575–3583

[87] Herberg S, Siedler M, Pippig S, et al. Development of an injectable composite as a carrier for growth factor-enhanced periodontal regeneration. Journal of Clinical Periodontology, 2008, 35(11): 976–984

[88] Song H Y, Esfakur Rahman A H, Lee B T. Fabrication of calcium phosphate–calcium sulfate injectable bone substitute using chitosan and citric acid. Journal of Materials Science: Materials in Medicine, 2009, 20(4): 935–941

[89] Zhang W, Liao S S, Cui F Z. Hierarchical self-assembly of nano-fibrils in mineralized collagen. Chemistry of Materials, 2003, 15 (16): 3221–3226

[90] Liao S S, Cui F Z, Zhang W, et al. Hierarchically biomimetic bone scaffold materials: nano-HA/collagen/PLA composite. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 2004, 69(2): 158–165

[91] Chen Z, Liu H, Liu X, et al. Injectable calcium sulfate/mineralized collagen-based bone repair materials with regulable self-setting properties. Journal of Biomedical Materials Research Part A, 2011, 99(4): 554–563

[92] Chen Z, Liu H, Liu X, et al. Injectable mineralized collagen-based bone repair materials. Journal of Controlled Release, 2013, 172(1): e148–e149

[93] Hu N M, Chen Z, Liu X, et al. Mechanical properties and *in vitro* bioactivity of injectable and self-setting calcium sulfate/nano-HA/collagen bone graft substitute. Journal of the Mechanical Behavior of Biomedical Materials, 2012, 12: 119–128

[94] Chen Z, Liu H, Liu X, et al. Improved workability of injectable calcium sulfate bone cement by regulation of self-setting properties. Materials Science & Engineering C: Materials for Biological Applications, 2013, 33(3): 1048–1053

[95] Lian X J, Liu H Y, Wang X M, et al. Antibacterial and biocompatible properties of vancomycin-loaded nano-hydroxyapatite/collagen/poly(lactic acid) bone substitute. Progress in Natural Science: Materials International, 2013, 23(6): 549–556

[96] Zalavras C G, Patzakis M J, Holtom P. Local antibiotic therapy in the treatment of open fractures and osteomyelitis. Clinical Orthopaedics and Related Research, 2004, 427: 86–93

[97] Jiang J L, Li Y F, Fang T L, et al. Vancomycin-loaded nano-hydroxyapatite pellets to treat MRSA-induced chronic osteomyelitis with bone defect in rabbits. Inflammation Research, 2012, 61(3): 207–215

[98] Joosten U, Joist A, Frebel T, et al. Evaluation of an *in situ* setting injectable calcium phosphate as a new carrier material for gentamicin in the treatment of chronic osteomyelitis: studies *in vitro* and *in vivo*. Biomaterials, 2004, 25(18): 4287–4295

[99] Cui X, Zhao C, Gu Y, et al. A novel injectable borate bioactive glass cement for local delivery of vancomycin to cure osteomyelitis and regenerate bone. Journal of Materials Science: Materials in Medicine, 2014, 25(3): 733–745

[100] Tsai Y F, Wu C C, Fan F Y, et al. Effects of the addition of vancomycin on the physical and handling properties of calcium sulfate bone cement. Process Biochemistry, 2014, 49(12): 2285–2291