

# Advances in the modification of injectable calcium-phosphate-based bone cements for clinical application

Xiao-Hui Wang<sup>1,2</sup>, Shuai-Jun Jia<sup>1</sup>, Ding-Jun Hao<sup>1</sup>

<sup>1</sup>Department of Spine Surgery, Honghui Hospital, Xi'an Jiaotong University, Xi'an, Shaanxi 710054, China;

<sup>2</sup>Health Science Center, Xi'an Jiaotong University, Xi'an, Shaanxi 710061, China.

Autografts, allografts, and xenografts are the most widely used methods to enhance effective reconstruction of bone defects, but natural donor bone has some deficiencies, such as limited supply, excessive damage of transplant-supplied-parts, constrained growth and high complication rates.<sup>[1]</sup> Bioactive bone replacement materials provide an alternative for bone defect repair and regeneration. Augmented injectable bone scaffold material applied to bone replacement therapies in orthopedics and dentistry has generated much discussion in bone tissue engineering for bone defect repair. Injectable cement materials currently in clinical use and those under research can be classified into three main categories: polymethylmethacrylate (PMMA), calcium-phosphate-based bone cements (CPCs), and calcium-sulfate-based bone cements (CSCs). Of these, the chemical components of CPCs are most similar to the inorganic components of bones. CPCs mimic the mineral phase of bone to formulate a natural lattice for bone tissue to promote natural bone ingrowth and remodeling.<sup>[2]</sup> Furthermore, in comparison with the most widely used PMMA cements, CPCs have a lower exothermic reaction temperature and promote better osteointegration. For these reasons, they have become one of the most promising bone repair materials.<sup>[3]</sup> Nevertheless, CPCs have some limitations: (1) Their slow degradation rate and lack of macroporosity to promote the formation of new bone make their osteoinduction capacity insufficient for clinical needs.<sup>[4]</sup> Modification efforts include fabricating porous/nano structures and/or drug delivery CPC composites to promote bone growth; (2) Another drawback is that the mechanical strength of CPCs cannot match the strength of human cortical bone; thus, strength modification is another research direction.

Because of the reduction in cell differentiation and the decrease in the potential of osteoblasts in osteoporotic

patients, the purpose of modifying bone replacement materials for these patients is to promote the differentiation of bone marrow mesenchymal stem cells (BMSCs) and to stimulate the potential of osteoblasts. The current approach is to use CPCs as a scaffold and to add BMSCs and/or osteogenic activity factors. In practice, specific studies rely on the strategy of combining bioactive-element-loaded CPCs (scaffold materials) with seed cells. Under this framework, the largest variable is the bioactive element, which may be platelet-rich plasma (PRP), bone morphogenetic protein-2 (BMP-2) or a metal. The introduction of bioactive elements into CPCs can not only amplify the inherent bioactivity but can also offset the side effects of these elements. For example, extracellular  $\text{Ca}^{2+}$  at low concentrations from CPCs can modulate the conformation of BMP-2, which can enhance Smad1/5/8 and mitogen-activated protein kinase (MAPK) signaling transduction and further stimulate the expression of osteogenic marker genes.<sup>[5]</sup> BMP-2 enhancement of osteoclast formation can cause bone or scaffold material resorption. CPC inhibits osteoclast-mediated resorption of cements, which can offset the increased osteoclast activity induced by BMP-2.<sup>[6]</sup> BMP-2, one of the most investigated osteogenic active substances, has also been loaded on CPC scaffolding for the acceleration of bone formation. Luo *et al*<sup>[7]</sup> aimed to implant recombinant human BMP-2-loaded CPC-composite BMSCs into nude mice; their findings preliminarily revealed the bone remodeling potential of CPCs and BMSCs as scaffold materials and seed cells in bone tissue engineering from the perspective of new bone-forming capability. Li *et al*<sup>[8]</sup> also confirmed the clinical potential of such modifications for bone repair from the perspective of promoting vascularization. PRP has also been applied as the bioactive element of the above framework. Growth factors in PRP can promote bone repair through local delivery of bioactive agents to influence critical physiological mechanisms, such as

Access this article online

Quick Response Code:



Website:

www.cmj.org

DOI:

10.1097/CM9.0000000000001092

**Correspondence to:** Dr. Ding-Jun Hao, Department of Spine Surgery, Honghui Hospital, Xi'an Jiaotong University, Xi'an, Shaanxi 710054, China  
E-Mail: haodjingjun@mail.xjtu.edu.cn

Copyright © 2020 The Chinese Medical Association, produced by Wolters Kluwer, Inc. under the CC-BY-NC-ND license. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

Chinese Medical Journal 2020;133(21)

Received: 27-05-2020 Edited by: Ning-Ning Wang

inflammation, angiogenesis, and extracellular matrix synthesis. Generally, incorporating multiple growth factors into scaffolding promotes greater angiogenesis and osteogenesis stimulation than the incorporation of a single growth factor; as the natural compounded liquid phase, PRP can play the role of a bioactive modification of CPCs in more aspects than BMP-2.

Some metals have also been found to have osteogenic activity and have been used in bone tissue engineering. Examples include magnesium, strontium and their compounds, prompting theoretical study into the development of metal-based CPCs. For example, Sr implanted in CPCs was shown to increase the expression levels of osteoblast-related genes and to promote the activity of alkaline phosphatase in an osteoblast-like cell line and in BMSCs.<sup>[9]</sup> Considering that the neurotransmitter calcitonin gene-related peptide can play a role in promoting bone healing and remodeling, this substance was added to Sr-modified CPCs by Liang *et al*<sup>[10]</sup> to improve the anti-osteoporosis bioactivity of Sr-modified CPCs in fracture patients with osteoporosis. The progressive exploration of the “X-Sr-CPC model” provides new options for the treatment of osteoporotic bone injury. Another alkaline earth metal that has attracted substantial attention is magnesium. Mg in CPCs was found to promote BMSC adhesion and osteogenic differentiation via an integrin-mediated mechanism.<sup>[11]</sup> In addition to revealing the partial association of magnesium and osteogenesis, that team evaluated their magnesium/calcium phosphate cements (MCPCs) in terms of promoting angiogenesis and found that the angiogenic potential of human umbilical vein endothelial cells was enhanced *in vitro* by the MCPC-mediated immune microenvironment.<sup>[12]</sup> Similar to the case of Sr-modified CPCs, Mg-modified CPCs can also regulate osteogenic genes mediated by growth factors or promote angiogenesis indirectly by inducing micro-environmental changes, but which of these alkaline earth metals has the better effect has not been assessed. Apart from alkaline earth metals, other bioactive metal ions also have the potential to accelerate bone healing in low doses. Among them, copper, cobalt, and chromium ions are the focus of much attention. Human essential trace elements, such as  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cr}^{2+}$ , and  $\text{Ga}^{3+}$ , have been introduced into CPCs at low doses to assess their bioactivity.  $\text{Cu}^{2+}$ -doped CPC has well established to be antibacterial, angiogenic, and capable of promoting BMSC differentiation and bone mineralization. Although introduction of  $\text{Cu}^{2+}$  into CPCs imparts notable bioactivity, cytotoxicity still occurs at high doses. In contrast,  $\text{Co}^{2+}$  has shown controversial results in related studies; therefore, this approach needs to be confirmed by more tests. The addition of  $\text{Cr}^{3+}$ -endowed CPCs was confirmed to positively affect bone formation, supporting both the proliferation of osteoprogenitor cells and the resorption of osteoclasts [Supplementary Table 1, <http://links.lww.com/CM9/A315>].

Owing to the limited accessibility of infected bone tissue to systemically administered drugs, localized antibiotic delivery is a common treatment for post-operative infections, as implemented by using bone cements as carriers for antibiotics drugs. Widely used PMMA is only marginally porous, and the diffusion of antibiotics into the surrounding bone tissue is limited to the outer surface of the cement. CSCs have also been reported as carriers for

antibiotics and are more favorable than PMMA because of their interconnected microporosity. CPCs not only have the advantages of CSCs but also are closer to the composition of bone. CPCs are mainly modified by metal or are used as carriers to deliver antibiotic drugs to exert anti-infection effects. Antibiotic-loaded bone cement treatment reduces dead space and achieves targeted drug delivery simultaneously. The remaining problems to be solved include the issue that the porosity and mechanical properties of CPCs can be considerably affected by introducing antibiotics; in addition, CPCs degrade slowly over a timescale unsynchronized with the release of loaded drugs. To mitigate these negative effects, CPCs can be modified with drug-loaded polymers, enhancing the mechanical properties and degradation of the cements through the acidic nature of the polymer decomposition. Similar to the case of the antibiotic-loaded polymer composite CPCs, studies on the antibacterial effects of metal-modified CPCs are also highly active, involving silver-, iron- or copper-modified CPCs, with confirmed outstanding non-cytotoxicity and anti-infective efficacy [Supplementary Table 1, <http://links.lww.com/CM9/A315>].

Similarly, we can use CPCs as carriers of anti-tumor drugs or radioactive materials and inject them into patients to achieve an anti-tumor effect [Supplementary Table 1, <http://links.lww.com/CM9/A315>]. These modifications, whether sustained release of anti-tumor drugs, magnetic tumor targeting, or radiological modifications, are intended to simplify the treatment process and reduce systemic side effects and pain in patients.

CPC scaffolding is expected to degrade at the same rate that new bone forms; therefore, when considering these two balancing factors, the biodegradability of CPCs has been of concern to researchers. Adding PLGA microspheres, which degrade faster than CPCs, as porogens for CPCs is the most commonly used modification strategy. However, PLGA degradation occurs hydrolytically, generating acidic degradation products, which can carry a risk of a localized inflammatory response. CPCs modified by other porogens such as saccharide microspheres have also reported, with the potential to neutralize the acid from PLGA to some extent.<sup>[13]</sup> Another way to accelerate degradation is to introduce organic phases that can be absorbed more quickly, such as allogeneic bone powder or autologous BMSC-PRP [Supplementary Table 1, <http://links.lww.com/CM9/A315>]. In addition, CPCs modified with the pre-eminent metal Sr have also been found to have excellent biodegradability and an osteoinductive accelerating capability in some tests for expanding clinical applications.<sup>[14]</sup>

Due to the limitations in mechanical properties and low fracture toughness resulting from brittleness, the application of CPCs in the treatment of vertebral compression or burst fractures is poor. To overcome this deficiency, many reinforcement strategies have been devised, such as adding fibers to form metallic or inorganic compounds, cross-linking and adjusting the hardening liquid. Many previous studies have indicated that certain nanoscale metal oxides, silk fibroin, chemically activated carbon fibers, chitosan fibers, and gelatinized starches can also enhance the compressive strength or anti-washout property of CPCs.

Incorporation of type I collagen (coI) into CPCs has been an active research area to enhance the mechanical properties of CPCs recently as well. It has also been confirmed that this technique can better promote bone integration while reinforcing CPCs.<sup>[15]</sup> However, as the primary organic phase of bone, crosslinked coI plays a substantial role in promoting cell adhesion through specific interactions with ligands and adhering cells; moreover, the compressive strength of CPCs is negatively modified after its addition.<sup>[16]</sup> Due to the indispensable bionic advantages of coI-CPCs and their inevitable shortcomings, certain other materials have been considered for secondary modification. For example, Sr doping in collagen-CPCs can provide more interlocked microstructures and a higher compressive strength. Regarding the compressive properties, the PLGA scaffolding is strong but too stiff; the fracture toughness of the plain collagen scaffold is favorable, but its stiffness is insufficient. Thus, introducing both into CPCs as a compound-modified additive is a strategy that reflects the complementary advantage effect. This specific idea can be used to offset the limitations associated with the preliminary modification [Supplementary Table 1, <http://links.lww.com/CM9/A315>].

Binary modifications, including both material and biological modifications, usually performed by adding additional ingredients after unitary modifications for a single primary purpose: achieving overall efficiency gains. In general, increasing the porosity of CPCs reduces their mechanical strength; hence, researchers have aimed to further strengthen the mechanical properties of CPCs. For example, the strategy of adding PLGA fiber to CPCs effectively solves the problems of low mechanical strength and low fracture toughness. Gunnella and Bungartz *et al*<sup>[17]</sup> successively added certain osteogenic active factors, including BMP-2, GDF5 protein, and a mutant GDF5 protein, and demonstrated that the bone formation capacity and angiogenic effect of PLGA fiber-reinforced CPCs were significantly improved. Some materials have more than one advantage as prospects for modifiers. Sr-modified CPCs have many significant features, such as anti-osteoporosis activity, biodegradation promotion, and high mechanical strength. Copper not only promotes the proliferation of osteoblasts but also has an inhibitory effect on bacteria. The collagen component simulates the organic phase in bone and improves the fracture toughness of CPCs [Supplementary Table 1, <http://links.lww.com/CM9/A315>]. If these modifiers were used as the protagonist of the initial modification, the next suitable modification would be to address their defects. The well-ordered modification model is supported by reliable experimental data and will soon be ready for clinical application.

### Funding

This work was supported by a grant from the Science and Technology Commission of Shaanxi Province of China (No. 2017ZDXM-SF-054).

### Conflicts of interest

None.

### References

- Bao XG, Shi MC, Hou CL, Xu GH. Recent progress in the construction of functional artificial bone by cytokine-controlled strategies. *Chin Med J* 2018;131:2599–2604. doi: 10.4103/0366-6999.244105.
- Li L, Lu H, Zhao Y, Luo J, Yang L, Liu W, *et al*. Functionalized cell-free scaffolds for bone defect repair inspired by self-healing of bone fractures: a review and new perspectives. *Mater Sci Eng C Mater Biol Appl* 2019;98:1241–1251. doi: 10.1016/j.msec.2019.01.075.
- Fang C, Lin Y, Sun J, Lin F. The chitosan/tri-calcium phosphate bio-composite bone cement promotes better osteo-integration: an in vitro and in vivo study. *Orthop Surg Res* 2019;14:162. doi: 10.1186/s13018-019-1201-2.
- Lodaso-Torrecilla I, van Gestel NAP, Diaz-Gomez L, Grosfeld EC, Laperre K, Wolke JGC, *et al*. Multimodal pore formation in calcium phosphate cements. *J Biomed Mater Res A* 2018;106:500–509. doi: 10.1002/jbm.a.36245.
- Zhang W, He H, Tian Y, Gan Q, Zhang J, Yuan Y, *et al*. Calcium ion-induced formation of  $\beta$ -sheet/turn structure leading to alteration of osteogenic activity of bone morphogenetic protein-2. *Sci Rep* 2015;5:12694. doi: 10.1038/srep12694.
- Montazerolghaem M, Rasmusson A, Melhus H, Engqvist H, Karlsson Ott M. Simvastatin-doped pre-mixed calcium phosphate cement inhibits osteoclast differentiation and resorption. *J Mater Sci Mater Med* 2016;27:81–83. doi: 10.1007/s10856-016-5692-7.
- Luo G, Huang Y, Gu F. rhBMP2-loaded calcium phosphate cements combined with allogenic bone marrow mesenchymal stem cells for bone formation. *Biomed Pharmacother* 2017;92:536–543. doi: 10.1016/j.biopha.2017.05.083.
- Li C, Jiang C, Deng Y, Li T, Li N, Peng M, *et al*. RhBMP-2 loaded 3D-printed mesoporous silica/calcium phosphate cement porous scaffolds with enhanced vascularization and osteogenesis properties. *Sci Rep* 2017;7:41331. doi: 10.1038/srep41331.
- Park JW, Kim YJ, Jang JH, Song H. Positive modulation of osteogenesis and osteoclastogenesis-related gene expression with strontium-containing microstructured Ti implants in rabbit cancellous bone. *J Biomed Mater Res A* 2013;101:298–306. doi: 10.1002/jbm.a.34433.
- Liang W, Li L, Cui X, Tang Z, Wei X, Pan H, *et al*. Enhanced proliferation and differentiation effects of a CGRP- and Sr-enriched calcium phosphate cement on bone mesenchymal stem cells. *J Appl Biomater Funct Mater* 2016;14:e431–e440. doi: 10.5301/jabfm.5000295.
- Zhang J, Ma X, Lin D, Shi H, Yuan Y, Tang W, *et al*. Magnesium modification of a calcium phosphate cement alters bone marrow stromal cell behavior via an integrin-mediated mechanism. *Biomaterials* 2015;53:251–264. doi: 10.1016/j.biomaterials.2015.02.097.
- Ding S, Zhang J, Tian Y, Huang B, Yuan Y, Liu C. Magnesium modification up-regulates the bioactivity of bone morphogenetic protein-2 upon calcium phosphate cement via enhanced BMP receptor recognition and Smad signaling pathway. *Colloids Surf B Biointerfaces* 2016;145:140–151. doi:10.1016/j.colsurfb.2016.04.045.
- Grosfeld EC, Smith BT, Santoro M, Lodaso-Torrecilla I, Jansen JA, Ulrich DJ, *et al*. Fast dissolving glucose porogens for early calcium phosphate cement degradation and bone regeneration. *Biomed Mater* 2020;15:025002. doi: 10.1088/1748-605X/ab5f9c.
- Zhu H, Guo D, Qi W, Xu K. Development of Sr-incorporated biphasic calcium phosphate bone cement. *Biomed Mater* 2017;12:015016. doi: 10.1088/1748-605X/12/1/015016.
- Hu MH, Lee PY, Chen WC, Hu JJ. Incorporation of collagen in calcium phosphate cements for controlling osseointegration. *Materials (Basel)* 2017;10:910. doi: 10.3390/ma10080910.
- Lee GH, Makkar P, Paul K, Lee B. Incorporation of BMP-2 loaded collagen conjugated BCP granules in calcium phosphate cement based injectable bone substitutes for improved bone regeneration. *Mater Sci Eng C Mater Biol Appl* 2017;77:713–724. doi:10.1016/j.msec.2017.03.296.
- Bungartz M, Kunisch E, Maenz S, Horbert V, Xin L, Gunnella F, *et al*. GDF5 significantly augments the bone formation induced by an injectable, PLGA fiber-reinforced, brushite-forming cement in a sheep defect model of lumbar osteopenia. *Spine J* 2017;17:1685–1698. doi: 10.1016/j.spinee.2017.06.007.

**How to cite this article:** Wang XH, Jia SJ, Hao DJ. Advances in the modification of injectable calcium-phosphate-based bone cements for clinical application. *Chin Med J* 2020;133:2610–2612. doi: 10.1097/CM9.0000000000001092