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Silicate-based bioceramics for periodontal regeneration

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Abstract

Periodontal disease is characterized by the destruction of the tissues that attach the tooth to the alveolar bone. Various methods for regenerative periodontal therapy including the use of barrier membranes, bone replacement grafts, growth factor delivery, have been investigated; however, true regeneration of periodontal tissue is still a significant challenge to scientists and clinicians. The focus in periodontal tissue engineering has shifted from attempting to recreate tissue replacements/constructs to the development of biomaterials that incorporate and release regulatory signals to achieve *in situ* periodontal regeneration. The release of ions and molecular cues from the biomaterials may help to unlock the latent regenerative potential in the body by regulating cell proliferation and differentiation towards different lineages (e.g. osteoblasts and cementoblasts). Silicate-based bioactive materials, including bioactive silicate glasses and ceramics have become the materials of choice for periodontal regeneration, due to their favourable osteoconductivity and bioactivity. This article will focus on the most recent advances in the *in vitro* and *in vivo* biological application of silicate-based ceramics, specifically as it relates to periodontal tissue engineering.

Introduction

Periodontitis is a bacterially induced inflammatory disease that affects the tissues supporting the tooth which consist of periodontal ligament, alveolar bone and cementum.[1] Periodontitis is a major cause of tooth loss in adults and is also associated with an increased risk of systemic morbidities such as cardiovascular diseases.[2] Conventional treatments for periodontitis such as scaling, root planning and open flap debridement can, to varying degrees, control further development of periodontal disease. Restoring the lost structure of the original periodontium, especially the re-attachment of the periodontal ligament to the newly formed cementum, remains an elusive goal.

Advances in regenerative medicine, stem cell biology and materials science have attracted increasing attention to periodontal tissue engineering, and biomaterials, in particular, play an integral role in the design of bioscaffolds and delivery systems. Over the past two decades, a range of biomaterials such as natural polymers (e.g., gelatin, collagen, and chitosan),[3-5] synthetic polymers (e.g., polyactides, polyglycolides, polyurethanes, and polycaprolactones),[6-8] ceramics (e.g., bioactive glass, calcium sulphate and calcium phosphate),[9-11] and more often their composites, have been intensively studied and applied in the field of periodontal tissue engineering.[12-14] Silicate-based bioceramics, as a new family of biomaterials, has proven to be an excellent material for bone tissue regeneration, and its preparation, mechanical strength, apatite mineralization and biological properties have been comprehensively reviewed in our previous viewpoint.[15] In this review, we will focus on the *in vitro* biological effect of silicate-based ceramics and their potential *in vivo* application in periodontal regeneration, as well as the future prospects.

Stimulation of in vitro cementogenic/osteogenic differentiation of PDLCs by silicate-based ceramics

Over the past decade, a new family of bioactive silicate-based ceramics with a wide range of compositions have been developed.[16, 17] Some of these silicate-based ceramics have profound pro-osteogenic and pro-angiogenic properties.[18, 19] One such bioceramic is akermanite ($Ca_2MgSi_2O_7$), a material that possesses a superior ability to promote cell proliferation and osteogenic differentiation of periodontal ligament cells (PDLCs), when compared to β-tricalcium phosphate (β-TCP).[20] Restoration of periodontal attachment requires not only regeneration of the alveolar bone but also new cementum formation to replace the diseased root surfaces contaminated with bacterial endotoxins. The ideal biomaterials for periodontal tissue engineering should favour both alveolar bone and cementum formation by stimulating the differentiation of progenitor cells toward osteoblastic and cementoblastic lineages. Recently, we have reported that the ionic extracts of a novel silicate-based ceramic powder nagelschmidtite (NAGEL: $Ca_7Si_2P_2O_{16}$) has excellent stimulatory effect on the cementogenic/osteogenic differentiation of PDLCs as shown in Figure 1.[21] The mRNA expression of cementum protein 1 (CEMP1), a local regulator of cementum metabolism and a key markers of the cementoblast phenotype,[22] increases significantly in PDLCs exposed to NAGEL in a concentration dependent manner. This indicates that these silicate-based biomaterials are capable of stimulating cementogenic/osteogenic differentiation of PDLCs for the regeneration of the lost periodontal apparatus. The exact mechanism of interaction between the ionic dissolution products from these inorganic materials and PDLCs are not fully understood and this has prompted considerable interest within the biomaterials community. We have shown that the Wnt/β-catenin signalling pathway can be activated when PDLCs are exposed to ionic extracts from bredigite ($Ca_7MgSi_4O_{16}$) [23] and that treatment with the Wnt/β-catenin inhibitor, cardamonin, can lead to a decrease in cementogenic/osteogenic

gene and protein expression. These results suggest that the ionic extracts of bredigite powder may stimulate cementogenic/osteogenic differentiation of PDLCs via the activation of the Wnt/β-catenin signalling pathway. More recently, we have reported that the zirconium modified silicate-based ceramics, baghdadite ($Ca_3ZrSi_2O_9$), possess specific *in vitro* cementogenic stimulation for PDLCs.[24] This study showed that baghdadite ceramic disks could support PDLC adhesion and proliferation, and significantly promote the expression of cementogenic/osteogenic markers. Ionic products from baghdadite powders had excellent pro-cementogenic/osteogenic effects, which may be related to the activation of Wnt/β-catenin signalling by the released Ca, Zr and Si ions. We also explored the effect of Si ions on the proliferation and differentiation of bone marrow stromal cells and these results showed that Si ions alone could stimulate osteogenic differentiation in these cells.[25] Our working hypothesis is, therefore, that released Si ions from silicate-based ceramics, play a key role in stimulating cementogenic/osteogenic differentiation of PDLCs, and that other ions, such as Mg, Zr, and P, may have synergetic effects.

Stimulation of in vivo cementogenesis/osteogenesis by silicate-based ceramics

The ability of silicate-based biomaterials to promote *in vitro* PDLC differentiation suggests that these particular materials have promising clinical potential. To demonstrate their *in vivo* application, these materials have been tested in beagle dogs. Our recent study showed that diopside (DIOP: $CaMgSi_2O_6$) could be a promising candidate for periodontal tissue engineering.[26] The DIOP scaffolds significantly induced bone and cementum regeneration in the periodontal tissue defects, compared to the conventional β-TCP scaffolds. The results suggest that silicate-based ceramics provide a desired microenvironment for root/periodontal tissue development, which enhances the reconstruction of the physiological architecture of a cementum/PDL-like complex. NAGEL scaffolds have also been recently synthesized and

tested *in vivo* in periodontal defects in beagle dogs [27]. The study suggests that this material can repair the lost periodontal tissues by inducing new bone and cementum growth (Figure 2). The significantly enhanced expression of bone sialoprotein (BSP) and osteocalcin (OCN) after the implantation of NAGEL scaffolds compared to β-TCP scaffolds, as demonstrated by inmmunohistochemical staining, further confirms that NAGEL scaffolds have excellent *in vivo* pro-cementogenic and pro-osteogenic effect. The *in vitro* data together with the *in vivo* study, suggests that the improved cementogenesis/osteogenesis may be directly related to the stimulatory effect of NAGEL scaffolds on the differentiation of PDLCs.

Conclusions and perspective

In this brief review we have highlighted the most recent *in vitro* and *in vivo* research findings of silicate-based ceramics related to the cementogenic/osteogenic differentiation of PDLCs for periodontal regeneration application. The molecular mechanisms may involve the release of Si ions that activate the Wnt/β-catenin signalling pathway in PDLCs, leading to the differentiation of PDLCs to cementoblasts and osteoblasts to generate cementum and alveolar bone respectively (Figure 3). The corresponding mechanisms need to be further clarified by investigating the interaction between PDLCs and silicate-based ceramics with tailored ion release kinetics, as well as selecting suitable large animal models. Further studies on the degradation mechanisms of silicate-based ceramics are needed to optimize the degradation rate *in vivo* to favour cell growth and differentiation. Other proposed experimental approaches are to introduce active metal ions into the ceramic network in order to exert the therapeutic effects of these ions, thus further improve the biological performance of the materials in terms of the host response. Comprehensive pre-clinical and clinical studies are needed to address some important issues related to periodontal regeneration such as the differences between animal models and human diseases, as well as the chronic infection of

periodontal tissues, before the silicate-based ceramics can be used clinically for periodontal regeneration.

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Legends

Figure 1. The effect of $Ca_7Si_2P_2O_{16}$ powder extracts on gene expression (ALP, Col I, Runx2, CEMP1) of PDLCs. **: significant difference ($p<0.05$) compared to blank control on day 14. Blank control: the medium supplemented with 10% FCS without addition of material extracts. (Reprinted with permission from Zhou Y. *et al.* Acta. Biomater., 2012, **8**, 2307-2316.).

Figure 2. $Ca_7P_2Si_2O_{16}$ scaffolds repaired the lost periodontal tissues in beagle dogs. Scale bars are 500, 200 and 100 mm, respectively. (Reprinted with permission from Wu C. et al. RSC. Adv., 2013, 3, 17843-17850.).

Figure 3. Silicate bioceramics stimulate the *in vitro* and *in vivo* osteogenesis and cementogenesis. Schematic illustration of the possible mechanism of silicate-based ceramics for periodontal tissue regeneration. The release of Si ions from the silicate-based ceramics may activate the Wnt/β-catenin signalling pathway in periodontal ligament cells, leading to the regeneration of bone and cementum.

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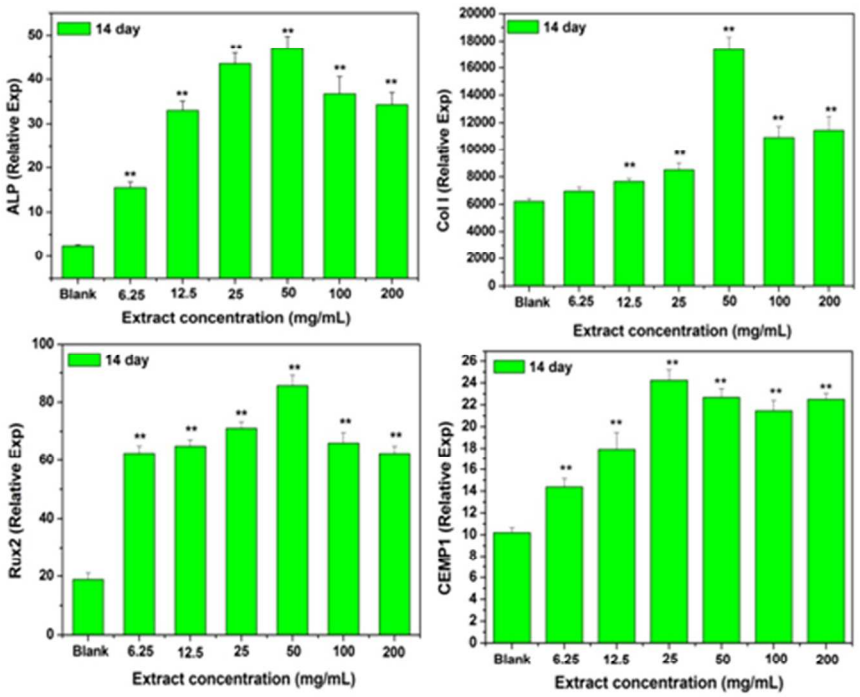

Figure 1. The effect of Ca7Si2P2O16 powder extracts on gene expression (ALP, Col I, Runx2, CEMP1) of PDLCs. **: significant difference ($p<0.05$) compared to blank control on day 14. Blank control: the medium supplemented with 10% FCS without addition of material extracts. (Reprinted with permission from Zhou Y. et al. Acta. Biomater., 2012, 8, 2307-2316.).
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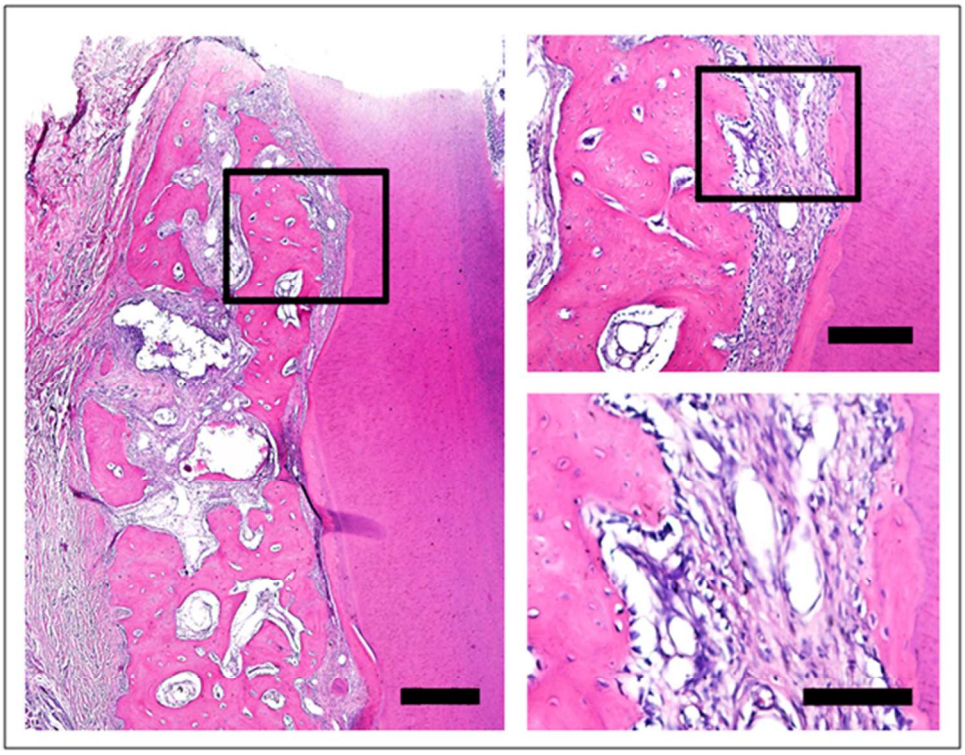

Figure 2. Ca7P2Si2O16 scaffolds repaired the lost periodontal tissues in beagle dogs. Scale bars are 500, 200 and 100 mm, respectively. (Reprinted with permission from Wu C. et al. RSC. Adv., 2013, 3, 17843-17850.).
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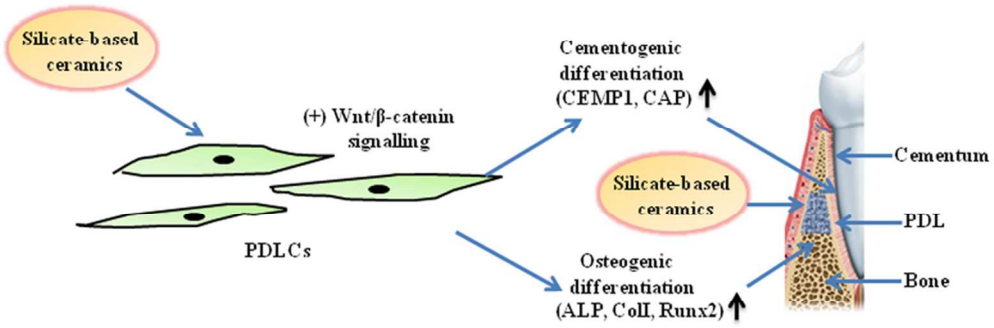

Fiure 3. Silicate bioceramics stimulate the in vitro and in vivo osteogenesis and cementogenesis. Schematic illustration of the possible mechanism of silicate-based ceramics for periodontal tissue regeneration. The release of Si ions from the silicate-based ceramics may activate the Wnt/β-catenin signalling pathway in periodontal ligament cells, leading to the regeneration of bone and cementum.
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