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# Osteogenesis and angiogenesis properties of dental pulp cell on novel injectable tricalcium phosphate cement by silica doped

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ABSTRACT

β-Tricalcium phosphate (β-TCP) is an osteoconductive material in clinical. In this study, we have doped silica (Si) into β-TCP and enhanced its bioactive and osteostimulative properties. To check its effectiveness, a series of Si-doped with different ratios were prepared to make new bioactive and biodegradable biocomposites for bone repair. Formation of the diametral tensile strength, ions released and weight loss of cements was considered after immersion. In addition, we also examined the behavior of human dental pulp cells (hDPCs) cultured on Si-doped β-TCP cements. The results showed that setting time and injectability of the Si-doped β-TCP cements were decreased as the Si content was increased. At the end of the immersion point, weight losses of 30.1%, 36.9%, 48.1%, and 55.3% were observed for the cement doping 0%, 10%, 20%, and 30% Si into β-TCP cements, respectively. In vitro cell experiments show that the Si-rich cements promote human dental pulp cell (hDPC) proliferation and differentiation. However, when the Si-doped in the cement is more than 20%, the amount of cells and osteogenesis protein of hDPCs was stimulated by Si released from Si-doped β-TCP cements. The degradation of β-TCP and osteogenesis of Si gives a strong reason to believe that these Si-doped β-TCP cements may prove to be promising bone repair materials.



## 1. Introduction

The suitable biomaterial for this purpose should be osteoconductive with mechanical and degradable properties matching that of bone formation. For absorbable bone cement applications, where the implant dissolves while the host tissue replaces it, calcium phosphate materials are widely used [1]. The autograft possesses the three essential elements that are required for good mechanical, osteoinductive and osteoconductive properties and it is currently evaluated the gold standard in this field. In clinical, calcium phosphate-based bone cements are extensively studied for reconstructive bone surgery due to its chemical similarity to nature bone tissue [2] and it can integrate into the physiological bone remodeling process [1,3]. Among calcium phosphate materials, hydroxyapatite and tricalcium phosphate (TCP) have been exhaustively analyzed because of their ability to bond to the bone tissue [4]. Therefore, the degradable bone substitute materials must be able to modulate both osteoblast and osteoclast functions in order to maintain the dynamics of bone remodeling [5,6]. Thus, the ability to regulate resorption properties is an added advantage for bone replacement materials [1].

Thus, the effect of silica doped into calcium phosphate material formulation has been investigated in several studies [7,8]. Silica-based materials have received a considerable amount of positive attention in recent years as these materials have better bioactivity than calcium phosphate-based materials [9,10]. In previous studies, silica has been proved to play an important role in bone formation, at least based upon this materials' Si ion release [11] and fast apatite formation ability [12]. Recently, the silica-based biomaterials has attracted great attention as a potential bioactive bone substitute material since it showed õexcellent bioactivity and osteostimulatory properties [1,11,13]. Our investigations proved that the bioactive Si ions released from silica-based materials could provide a preferential extracellular environment for directing osteogenic [13] and odontogenic [14] differentiation and enhance angiogenesis [15] properties of human dental pulp cells (hDPCs). Furthermore, the Si ion released from the silica-based cement

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**Table 1**
Setting time and diametral tensile strength of various amounts of Si doped in β-TCP mixed with $ddH_2O$.

| Sample | Setting time (min) | Diametral tensile strength (MPa) |
|---|---|---|
| Si0 | 78 ± 3[a] | 1.68 ± 0.11[e] |
| Si10 | 60 ± 4[b] | 1.81 ± 0.14[e] |
| Si20 | 43 ± 2[c] | 2.25 ± 0.19[f] |
| Si30 | 28 ± 2[d] | 2.37 ± 0.21[f] |

Values are mean ± standard deviation. Ten samples were measured for each data.
Values not sharing a common letter are significantly different at $p < 0.05$.

can abrogate and modulate cell through dose-dependent in vitro RANKL-mediated osteoclastogenesis [6]. In vivo study also showed that silica-based materials inhibited early inflammatory responses [16] and tissue changes during implantation tests in animals [17]. In addition, the silica-based materials stimulated significantly bone formation comparing with the traditional calcium phosphate biomaterials [18]. Moreover, Si is an element that is known not only to stabilize the β-TCP form, but also to enhance the bioactivity and the osteogenic potential [19,20].

In previous study, we regulated the mechanical and biological properties of TCP by mixing calcium silicate, and the bioactivity, degradation, and cell behavior of TCP/CS composites were better than pure TCP. Thus, we believe that the Si ion not only regulates mechanical properties but also promotes cell behavior. Therefore, we attempted to dope Si into β-TCP and evaluated its mechanical and biological properties. In this study, Si-doped β-TCP cements with varied ratios were prepared so that we could observe the changes in physiochemical properties, in vitro degradation behavior, osteogenesis, and angiogenesis activity in various ratios of Si-doped. It is our hope that this knowledge may help in the design of optimal biomaterials for bone regeneration.

## 2. Materials and methods

### 2.1. Preparation of Si doped β-TCP cement

The reagent grade $SiO_2$ (Sigma-Aldrich, St. Louis, MO) and β-TCP (Sigma-Aldrich) powders were used as matrix materials. The particle size was approximately (~80%) between 1 and 5 μm. The $SiO_2$ and β-TCP mixtures were sintered at 1400 °C for 3 h using a high-temperature furnace and then ball-milled in ethyl alcohol using a centrifugal ball mill (S 100, Retsch, Hann, Germany) for 6 h. The specimen codes "Si10", "Si20" and "Si30" were used to indicate cement containing $10SiO_2$/90β-TCP, $20SiO_2$/80β-TCP and $30SiO_2$/70β-TCP (in wt.%), respectively. The powder was mixed using a liquid/powder ratio of 0.25 mL/g. After mixing with liquids (1 M $Na_2HPO_4$), the cements were molded in a Teflon mold (diameter: 6 mm, height: 3 mm). The cement quantities were such that they fully covered each well of the 24-well plate (GeneDireX, Las Vegas, NV) to a thickness of 2 mm for cell experiments. All samples were stored in an incubator at 100% relative humidity and 37 °C for 1 day of hydration.

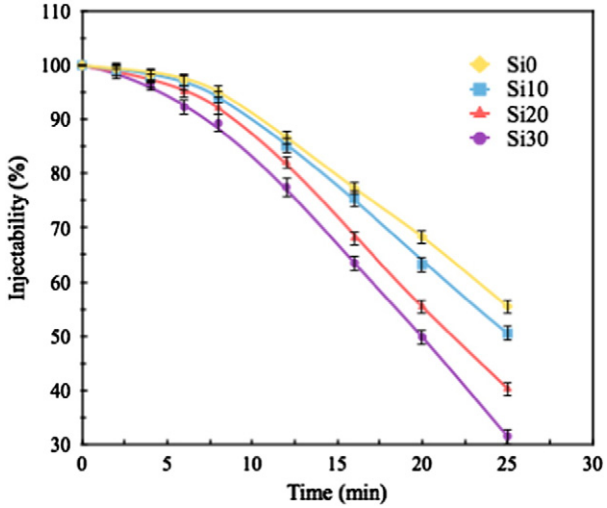


**Fig. 1.** Injectability of different Si-doped TCP pastes after versus setting time.

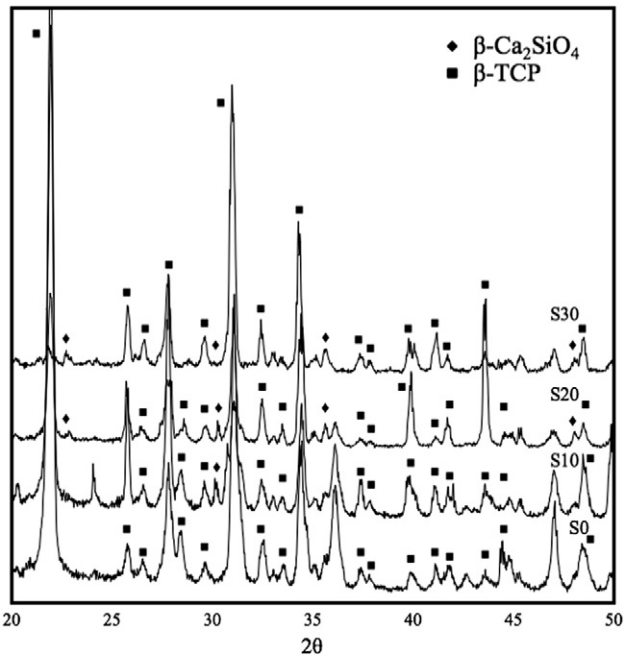


**Fig. 2.** XRD patterns of cements have different Si-doped ratios after hydration.

### 2.2. Setting time and strength

After the powder was mixed with liquid, the cements were placed into a cylindrical mold and stored in an incubator at 37 °C and 100% relative humidity for hydration. The setting time of the cements was tested according to standards set by the International Standards Organization (ISO) 9917-1. For evaluation of the setting time, each material was analyzed using Gilmore needles (456.5 g), and recorded when the needle failed to create a 1-mm deep indentation in three separate areas.

After being taken out of the mold, the specimens were incubated at 37 °C in 100% humidity for 1 day. The diametral tensile strength (DTS) testing was conducted on an EZ-Test machine (Shimadzu, Kyoto, Japan) at a loading rate of 1 mm/min. The maximal compression load at failure was obtained from the recorded load–deflection curves. The ten specimens were examined for each of the materials.

### 2.3. Injectability

The injectability of Si-doped TCP cement paste was considered by pressing 2.5 g of as-prepared paste through a 5 mL syringe with the opening needle with the diameter of 2.0 mm by hand, suggesting that injection by hand possessed even slightly lower standard deviations than injection by machine with preset load. After hydration at 37 °C in a 100% relative humidity for different incubation times, the paste was extruded from the syringe until it was unable to be injected. The weight of the paste injected through the syringe was measured. The injectability was calculated as: $I = m_{injected} / m_{initial} \times 100\%$, where I is the injectability, and $m_{injected}$ and $m_{initial}$ are the weight of the paste injected through the syringe and the paste initially contained in the syringe. All values were the average of ten tests performed for each group.