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Leading Opinion

# Silicon-substituted calcium phosphates – A critical view☆,☆☆

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ABSTRACT

Nowadays, the scientific community widely accepts the statement that silicon-substituted calcium phosphates have better biological properties compared to pure calcium phosphates. For example, a review published in this journal in 2007 started with the sentence "Silicon (Si) substitution in the crystal structures of calcium phosphate (CaP) ceramics such as hydroxyapatite (HA) and tricalcium phosphate (TCP) generates materials with superior biological performance to stoichiometric counterparts" [1]. A critical look at published articles demonstrates that this sentence is controversial and somehow misleading, because there is no experimental evidence that Si ions are released from Si-substituted calcium phosphates at therapeutic concentrations, and because there is no study linking the improved biological performance of Si-substituted calcium phosphates to Si release. The aim of this article is to explain this statement in more details.



## 1. Manuscript structure

To do so, the manuscript is divided into several sections, each dealing with a different topic. In the first section, a brief history is presented to explain why Si raised the interest of researchers. The aim of the second section is to show that there is presently no clear data supporting the conclusion that Si is released from Si-substituted hydroxyapatite (Si-substituted HA) in vivo, and that no attempt has been made to match the released Si amounts with biological performance. In the third section, several mechanisms that can potentially explain the biological effect of Si substitution in calcium phosphates are presented and discussed. The fourth section attempts to demonstrate that there are at the moment very few in vivo studies demonstrating a positive effect of Si on the biological response of calcium phosphates. The practical significance of a positive biological effect of Si substitution is discussed and questioned in the fifth section. Finally, a brief conclusion is presented.

## 2. History

In 1970, Carlisle [2] suggested that "Silicon may be allied to the initiation of mineralization of preosseous tissues". Since then, numerous articles have confirmed the metabolic effect of Si on bone [1], so it can be considered as an accepted fact. The positive effect of Si on bone metabolism has raised the interest of research groups working on Si-containing bone graft substitutes, in particular those working with bioglass [3]. In the 1990's, researchers started making efforts to develop silicon-substituted calcium phosphates (also named "silicated calcium phosphate" and "silicate-substituted calcium phosphate") [4–6]. Since then, two groups of researchers have produced an impressive number of studies: the group of professor Bonfield working on silicon-substituted HA and the group of professor Sayer working on silicon-substituted α-tricalcium phosphate. Research efforts in the field of Si-substituted calcium phosphates have been summarized in several recent reviews [1,7,8]. The summary of the general conclusions of these studies is that "silicon-substituted calcium phosphates have better biological properties compared to pure calcium phosphates" [1].

## 3. Si release from Si-substituted HA?

The various groups working on silicon-substituted calcium phosphates have assumed that silicon-substituted calcium phosphates are resorbed during implantation, hence leading to Si ions release, and that through this release, cells are positively influenced. Unfortunately, barely any experimental evidence has been provided to support this chain of action. Specifically, very little has been done

☆ Answer to the review article of Pietak et al.: "Silicon substitution in the calcium phosphate bioceramics" (Biomaterials, 28 (2007) 4023–32).

☆☆ *Editors Note*: This paper is one of a newly instituted series of scientific articles that provide evidence-based scientific opinions on topical and important issues in biomaterials science. They have some features of an invited editorial but are based on scientific facts, and some features of a review paper, without attempting to be comprehensive. These papers have been commissioned by the Editor-in-Chief and reviewed for factual, scientific content by referees.

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(i) to quantify the resorption of Si-substituted calcium phosphates, (ii) to measure the in vivo Si release, and (iii) more importantly to demonstrate that the positive biological effects seen when using Si-substituted calcium phosphates are due to Si ion release and not to other effects such as topographical effects or the release of Ca ions. Looking at the resorption of Si-substituted calcium phosphates, solubility data suggests that Si-substituted tricalcium phosphate is resorbable through a higher solubility [1] and hence can release Si ions. This appears however unlikely to happen for Si-substituted HA since Si-substituted HA is chemically very similar to HA and since HA is completely insoluble in body fluids [9–11] and hence known to be practically inert in vivo [12]. For example, Balas et al. [13] demonstrated that Si-substituted HA did not dissolve during more than two weeks in a solution containing initially about 10 times less Ca ions than in serum and about 1000 times less phosphate ions than in serum. In two in vivo studies devoted to Si-substituted HA [14,15], resorption was not quantified. Nevertheless, TEM examinations performed on samples retrieved from these two studies revealed the presence of dissolution pits at crystal surfaces [16,17]. However, no quantification and statistical analysis were performed. In a recent study [18], the in vivo behaviour of silicated calcium phosphate (SiCaP) was compared to that of dense calcium sulphate, and highly porous β-tricalcium phosphate. SiCaP demonstrated superior bone formation compared to the two other materials. Importantly, some resorption was reported. However, there was no XRD data provided in the study to indicate the nature of the material (e.g. β-TCP, α-TCP, HA?), no clear denomination (e.g. hydroxypatite), and no trade name that would have helped to identify the product and as a result the material composition. Furthermore, the samples were implanted in a mechanically loaded area in an osteochondral site. As mechanical loading has been suggested to affect the in vivo behaviour of bone substitutes [19] and as synovial fluids contain compounds promoting osteoclastogenesis [20], it is likely that the results did not reflect the behaviour that would be seen in a typical bone graft substitute application. It is also likely that resorption was enhanced due to mechanical loading and the proximity of synovial fluids.

Despite the fact that some of the in vivo studies mentioned herein detected material resorption, none of these studies either looked at Si release, or attempted to determine the rate of Si release or discussed the Si levels that should be reached to obtain a therapeutic effect. Also, none of the studies discussed the possibility that not Si release but Ca release could be responsible for the positive outcome, since calcium ions are known to have a strong effect on bone cells, in particular osteoblasts [21]. At this point, it is important to mention that in the drug delivery field, it is a standard requirement to look at the release rate, availability and therapeutic level of a drug when discussing the adequacy of a drug delivery system.

Most of the argumentation to support the active role of Si on the biological response is based on in vitro data. For example, Porter et al. [16] claimed that the incorporation of Si in Si-substituted HA enhanced HA solubility and dissolution rate. But no quantitative data was provided. Contrarily, Botelho et al. [22] did quantify calcium and phosphate release after incubating HA granules with mononuclear cells (osteoclast precursors). These authors observed a significant but very limited increase of Ca and phosphate release at long incubation time of 1.5% Si-substituted HA, but since the authors did not indicate the amount of granules present in the culture medium, it is not possible to evaluate the degradation rate. Moreover, no attempt was made to measure Si release. Guth et al. [23] studied the dissolution rate of Si-substituted HA (commercial product) and in particular measured Si release, but the authors did not mention in which medium they made these tests. In another study, Botelho et al. [24] looked at the dissolution rate of HA and 1.5% Si-substituted HA in simulated body fluid (SBF), either in static or in dynamic conditions. Unfortunately, the authors did not provide the buffer composition and again did not measure Si release (only Ca and phosphate release). Si-substituted HA was observed to induce an earlier precipitation of HA after several days of incubation in SBF compared to pure HA, but neither standard deviation nor statistical analysis were provided. Si release was measured with XPS, which led the authors to conclude that there was a "preferential ionic release of Si" but XPS accuracy was neither assessed nor discussed.

One of the mechanisms put forward to explain the higher resorption rate of Si-substituted HA compared to pure HA is the reduction of the grain size [25–27]. It is indeed known that solubility increases with a decrease of particle (or grain) size. According to Wu and Nancollas [28], the increase of solubility is given by:

$$\ln\left(\frac{S_r}{S_\infty}\right) = \frac{2M\gamma_{SL}}{\rho\nu RT}\cdot\frac{1}{r}$$

In this equation, $S_r$ is the solubility of the particle with radius, $r$, $S_\infty$ is the normal solubility (of a plane surface), $M$ is the molar ratio (502 g/mol), $R$ is the gas constant (=8.3144 J/mol K), $T$ is the temperature (=310 K), $\nu$ represents the number of moles of ions formed from one mole of electrolyte (=9), and $\gamma_{SL}$ is the interfacial tension (=0.01 J/m$^2$). Even though the value of the interfacial tension varies from study to study [29], a 10% solubility increase is only reached for particle sizes in the order of 1–3 nm. So, since the grain sizes disclosed in articles devoted to Si-substituted HA are in the order of 1 μm, a change of grain size cannot be the cause of a solubility change.

## 4. By which mechanisms could Si substitution positively influence calcium phosphate biological properties?

Despite these criticisms, there is converging evidence that the addition of Si to calcium phosphate (α-tricalcium phosphate or HA) modifies the in vivo behaviour of Si-substituted calcium phosphates. For example, Patel et al. [15] implanted granules of pure HA and Si-substituted HA in a rabbit model, and observed an increase of bone ingrowth and bone–implant coverage. Similar results were obtained by Hing et al. using porous scaffolds [14]. To explain these results, several mechanisms, either active or passive, can be proposed. When active mechanisms are involved, Si ions are released and are "seen" by cells, hence affecting their metabolism. When passive mechanisms are involved, the presence of Si in the HA structure causes chemical or topographical changes at the surface that eventually lead to a change of the biological response. In the latter case, it is not the chemical nature of Si that is responsible for the response, but rather chemical or physico-chemical changes provoked by the presence of Si. In other words, in such studies authors are misleading the readers when they mention that silicon modifies the metabolic activity of bone cells since cells do not see Si at all due to the absence of material dissolution and hence Si release.

As previously mentioned, several passive mechanisms have been proposed in the scientific literature to explain the effect of Si substitution such as a change of grain size [25–27], a change of protein conformation at the material surface [30], or a change of surface topography, since surface topography affects the cellular behaviour [31], and since a decrease of grain size was observed with an increase in Si-substitution degree [27,32]. For example, Gomi et al. [33] compared the osteoclastic response of a smooth sintered HA surface to two roughened HA surfaces. More TRAP+ cells and multinucleated TRAP+ cells were found on the roughened surfaces, whereas more resorption lacunae were found on smooth surfaces. To our knowledge, none of the studies devoted to Si-substituted calcium phosphates have looked at surface topography.

Instead of discussing passive mechanisms to explain the effect of Si substitution on the biological response of Si-substituted calcium

phosphate compounds, it appears more interesting to look at the possibility that an active mechanism, i.e. Si release, is responsible for these changes. Herein, it has been shown that it is very unlikely that resorption occurs of Si-substituted HA due to the very low solubility of this compound. However, Si release could occur if Si is not included in the crystallographic structure of Si-substituted HA, but is present within the HA as fairly soluble compound. In fact, this aspect has been looked at very carefully by various research groups [13,24,35]. For example, Balas et al. [13] observed the polymerization of silicate species at the surface for a substitution degree larger than 1.6%. Similar results were also observed by Botelho et al. [24]. Since most authors have worked with lower degrees of silicon substitution (i.e. <1.6%), Si should have been present within the HA structure. However, a very recent study published by Gasquères et al. [35] has raised some doubts on this interpretation. Interestingly, the presence of fairly soluble Si compounds could explain the observation made by Porter et al. [16] and Botelho et al. [24] that preferential release of Si occurs.

## 5. Si-substituted calcium phosphates in bone healing

According to Pietzak et al., the "superior biological performance of Si–HA and Si–TCP implant materials has been well documented" [14,15,22,34,36–38]. A look at these documents contradicts this statement.

In [38], a sintered HA and an Si-substituted calcium phosphate were implanted in an immuno-deficient mouse. The Si-substituted calcium phosphate fared better than HA, but since many parameters were changed between the two bone graft substitutes, in particular the composition and hence degradation rate, it is impossible to state that the difference came from the presence of Si. In [14], Hing et al. looked at the in vivo behaviour of five silicon-substituted HA materials containing the following Si content: 0, 0.2, 0.4, 0.8 and 1.5%. The results showed some significant differences between the various groups, but no clear picture came out of the study: none of the investigated responses such as mineral apposition rate or bone volume, was a simple function of the Si content. Even though the solubility of the five different groups was not measured, it was mentioned that "HA granules substituted with 0, 0.8 and 1.5 wt% Si implanted for 6 and 12 weeks demonstrated localized dissolution of all apatites at a rate and extent linked to the level of substitution". As it is well-known that a resorbable calcium phosphate ceramic such as β-TCP generates a different response than a non-resorbable ceramic such as HA, and as it is also known that Ca ions have an profound effect of bone cells [21], it is not possible based on the study of Hing et al. to conclude that the different biological response of Si-substituted HA is due to Si release: it could also have been due to Ca release.

In [37], an Si-substituted calcium phosphate bone graft substitute was tested in vivo in a sheep model. This study revealed that the Si-substituted calcium phosphate was resorbable (after 2 years, the samples were "essentially completely resorbed") but since the material was not compared to any other material, it is not possible to conclude that Si-substituted calcium phosphate present a "superior biological performance".

Several cell culture studies have been performed as well [22,34]. For example, in [22], human osteoblasts were cultured on HA, 0.8% Si-substituted HA and 1.5% Si-substituted HA. The three materials were found to behave differently (at a significance of $p < 0.05$), but no clear pattern could be found: even though the 0.8% Si-substituted HA appeared to perform slightly better than pure HA and 1.5% Si-substituted HA, it was not at all times the case. The authors concluded that the "human osteoblasts are affected by the presence of silicon in the HA substrate", but did not measure Si release. In [34], no Si release was measured either, but at least the Ca and phosphate release were measured. Ca concentration in the cell culture medium was slightly but significantly increased over time, which led the authors to conclude that the 1.5% Si-substituted HA sustained a "higher osteoclastic resorption activity" than pure HA. However, no other markers for osteoclast activity were measured and only the results obtained at 4, 11 and 19 days were presented, despite the fact that "the medium was changed every 3 days for a period of 21 days and analyzed for calcium and phosphate (…)".

In [15], the authors compared the in vivo behaviour of HA and Si-substituted HA and observed significantly more bone ingrowth and more bone/implant coverage with Si-substituted HA. However, this study does again not demonstrate conclusively that the effect of Si substitution is due to the active biological effect of Si, because there is no evidence that resorption occurred and because various other factors varied between the two materials. For example, the observed differences could have been due to a passive mechanism, for example a change of surface topography or surface area. The surface topography was not measured and despite the fact that the authors stated that the surface area was "similar", Si-substituted HA was shown to have a larger surface area than pure HA: 2.63 against 2.36 "sq $mg^{-1}$" (the units are not clearly stated by the authors; the authors did not provide any standard deviation value).

In [18], osteochondral defects were made through the articular surface into the subchondral bone of the femoral condyle of New Zealand white rabbits and filled with cylindrical pellets of three materials: dense calcium sulphate, ultraporous β-tricalcium phosphate, and porous silicated calcium phosphate. Resorption of the silicated calcium phosphate material was observed between week 6 and week 12 of implantation, suggesting that Si ions were released during that process, but it is not possible to know whether the "Silicate-substituted calcium phosphate" mentioned in the article corresponds to a "silicate-substituted HA" because no XRD data were provided and no trade name that could help identifying the product was mentioned. Furthermore, the model that was used (osteochondral defects) is not good to prove the ability of the material to act as bone substitute, because synovial fluids contain compounds promoting osteoclastogenesis [20]. Furthermore, cyclic loads are known to affect the in vivo behaviour of bone substitutes [19].

In [36], different biological behaviours were obtained with different materials. Between materials, not only the composition, but also the microstructure/porosity and the solubility varied. So, it is not possible to know whether the effects were associated with Si release or with a general change of material properties. Puzzlingly, the dissolution rate of all apatite compounds tested in this article and expressed as Ca release was higher than that of β-TCP. Based on the respective solubility of HA and β-TCP, the opposite result should have been measured, suggesting the presence of amorphous phase in the HA samples (the authors did not evaluate the amorphous content in the powders).

The last study mentioned by Pietzak et al. [1] to support the statement that "superior biological performance of Si–HA and Si–TCP implant materials has been well documented" is an in vivo study of Mastrogiacomo et al. [37]. Since this study was only devoted to one material, namely an Si-substituted calcium phosphate, no conclusion can be drawn. So, to conclude this brief overview of the biological performance of Si-substituted calcium phosphates, Si-substituted calcium phosphates have been found to perform very well in vivo, but there is no data supporting the statement that Si–HA and Si–TCP have a "superior biological performance".

## 6. Significance of Si substitution for bone healing

The various studies reviewed here suggest that Si substitution in calcium phosphate materials positively affect their biological response. However, at present, none of the studies published on the

topic demonstrate the "superior biological performance" of Si-substituted calcium phosphates. Furthermore, it has not been shown how Si can act on the in vivo behaviour of Si-substituted calcium phosphates. For this purpose, Si release should have been measured in vivo or at least inferred from resorption measurements and related to therapeutic concentrations. It is unlikely that Si concentrations would spontaneously reach adequate therapeutic levels. For Si-substituted HA, the situation is even more complicated because there is no significant evidence showing in vivo resorption. Even though resorption would occur, the use of Si-substituted HA does not appear to be very interesting. Indeed, calcium phosphate bone graft substitutes are fragile and mechanically weak [39]. So, it appears that the best strategy to heal a bone defect is to favour a rapid turnover from a defect into mature bone. As mentioned by Botelho et al. [22], this implies that the bone graft should be "completely replaced by new bone". However, Si-substituted HA is very poorly resorbable. So, the use of Si-substituted HA does not allow an adequate transduction from a defect to mature bone. More resorbable calcium phosphates, such as β-TCP or biphasic calcium phosphates consisting of β-TCP and HA mixtures appear much more attractive.

## 7. Conclusion

The aim of this article was to demonstrate that despite claims made in numerous articles, it is not presently clear if and how Si substitution positively influences the biological response of Si-substituted calcium phosphates, and in particular Si-substituted HA. Presently, several explanations can be put forward: an active effect as claimed by researchers and companies involved with Si-substituted calcium phosphate or a passive effect involving for example calcium release, a change of surface chemistry, or a change of surface topography. As a final note, the present thorough analysis of the data published on Si-substitutes calcium phosphates leads to the conclusion that our perception of the role of resorbable calcium phosphate bone substitutes should be changed: these materials are not simply "bone graft substitutes" but are truly drug delivery systems. This implies that all ions released during bone substitute resorption should probably be considered as drugs. It also implies that the release rate, the availability and therapeutic levels of each ion should be considered (or at least kept in mind) when discussing in vivo results.

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