Silicates and Bone Fusion

Walid Waked, MD and Jonathan Grauer, MD
Department of Orthopaedics and Rehabilitation
Yale University School of Medicine, New Haven, CT

The development of new bone graft substitutes in the orthopedics arena is becoming more focused on harnessing patients' inherent osteoconductive, osteoinductive and osteogenic properties. Simultaneously, surgeons are changing their operating procedures to try to avoid the potential morbidity associated with autograft harvest, particularly from the iliac crest. Porous ceramic materials are one potential class of bone graft material. Maximizing structural and chemical properties are important objectives.

Silicon has been demonstrated to be important for bone formation with dietary intake studies. Bone integration with silicate-substituted products such as Actifuse™ Synthetic Bone Graft has been shown to facilitate bone formation, providing solid evidence of its place as a new class of bone graft material.

I. Properties of bone graft materials

In orthopaedics, bone grafting is used to promote bone formation in osseous defects created by trauma or surgical intervention. Graft material may be used to fill bony defects or facilitate union or fusion (Figure 1). Bracing, casting, external fixation, or internal instrumentation may temporarily stabilize a region during the repair process, but it is the biological process of new bone growth and fusion which leads to lasting results.

There are a number of relevant terms which bear definition. Osteoconductive materials provide scaffolding onto which new bone can be formed, osteoinductive materials induce precursor cells down a bone forming lineage, osteopromotive materials induce the proliferation of osteoblasts and osteogenic materials have the ability to directly provide bone forming cells. The ideal bone grafting material will possess all of these properties.

Autograft is bone taken from the patient's own body and transported to the site of interest. This is the most common form of bone graft and its use has weathered the test of time. It possesses all of the characteristics of ideal bone graft described above. As such, it continues to be considered the "gold standard" grafting material. Nonetheless, autograft is associated with defined morbidities. These include long-term pain, infection, or fracture, at the donor site. Furthermore, the quantity of autograft is limited and, depending upon the age and health of the patient, it may not yield bone formation to the degree desired.

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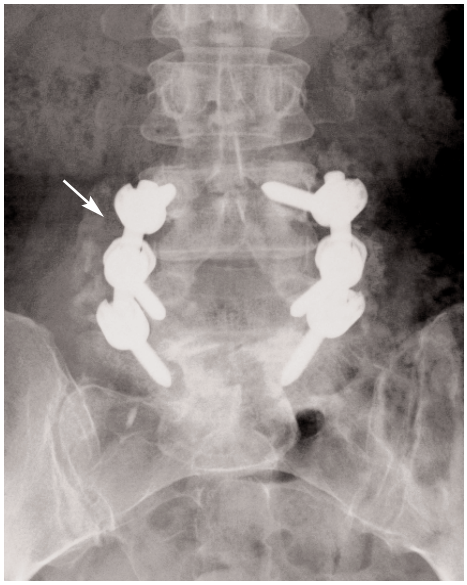
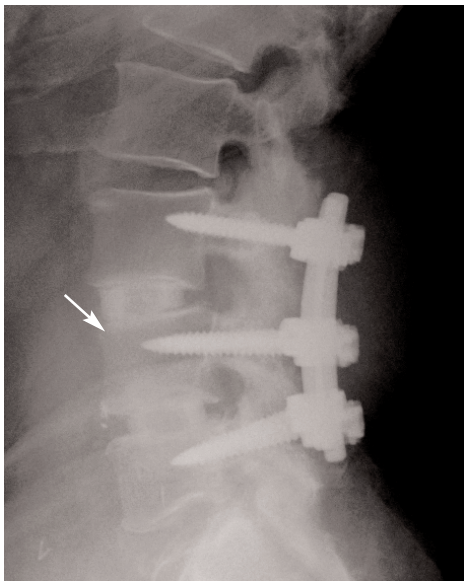
Consequently, significant efforts have been, and continue to be, given to developing and characterizing



A.



B.

Figure 1
Examples of two different patients with instrumented lumbar fusions.

A. Demonstrates bone formation in the posterolateral gutters (arrow).
B. Demonstrates anterior interbody structural grafts (arrow).

potential bone graft alternatives/supplements. For example, allograft using processed cadaveric bone. Particularly in the United States, this is frequently used in one of a number of different preparations ranging from fresh-frozen to demineralized bone matrix (DBM). Each of these formulations has specific properties, but none can reproduce all of the desirable characteristics of autograft and lower fusion rates have been found in many applications.[1] Further, there are ethical considerations attached to the use of allograft in some cultures.

Other potential bone graft materials have also received significant attention. Materials can be taken from patients themselves. These include bone marrow aspirates or platelet product isolates. Materials can be derived from molecular techniques such as recombinant human bone morphogenetic proteins (rhBMPs). Alternatively, synthetic bone graft substitutes are another class of potential bone graft materials. This class includes a number of materials with a wide range of chemistries and structural morphologies. The ideal synthetic bone graft substitute would be structurally similar to bone, easy to use, yield high fusion rates, and be cost-effective.[2]

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II. Synthetic bone graft materials

a. Features

There are several key design considerations for synthetic bone graft materials. Of course, they must be biocompatible and most are osteoconductive. As synthetic materials, their supply is not limited and their composition is uniform. However, osteoinductive and osteogenic components must be added to the grafting material or recruited from the host fusion bed.

The properties and effectiveness of synthetic bone graft materials, nonetheless, are quite varied. According to the situation at hand, the surgeon must choose the type of bone graft material that is most suitable for the desired situation. For example, these materials are resorbed at different rates. In some applications, such as a long bone fracture, one might desire complete resorption of a synthetic material in a timely fashion, but this may not be true in other scenarios such as anterior spinal reconstructions. Handling characteristics also vary significantly. Injectable materials are particularly suited to certain applications, e.g. distal radius fractures, but not others. Additionally internal morphologies such as macro and microporosity, degree of interconnectedness and the nature of the synthetic material all vary and result in differing host bone responses.

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In order to choose the correct synthetic material for the grafting application, one must understand the different types and forms of synthetic materials. Many of these fall under the description of ceramics — nonmetallic, inorganic materials that exhibit strength and stiffness.

b. Bioceramics

The prototypical bioceramic is hydroxyapatite (HA), a hydrated calcium phosphate similar in crystalline structure to the mineral of bone. This osteoconductive bioceramic has been studied extensively since the 1970s. As a bioactive bone material, it can bond to the host bone, as opposed to bioinert materials such as polymers, metals, and inert ceramic materials. However, there are significant differences between synthetic HA and that formed by the body itself. Not only are there crystalline differences, but the highly pure, manufactured form lacks many of the inorganic molecules and trace elements incorporated into the form produced *in vivo*.

The differences between endogenous HA and exogenous HA lead to altered resorption rates with the latter remaining at the site of repair for prolonged periods. The manufactured form may remain at the site of implantation for years or even decades.[3,4] In certain circumstances, this prolonged resorption may be advantageous, but in most situations it is preferable to have a graft material gradually replaced by host bone.

As with other implanted materials, calcium phosphates are resorbed by several mechanisms. A portion of the material may dissolve into surrounding biologic milieu. This would ideally coincide with the natural processes of bone healing and creeping substitution. Alternatively, cell-mediated processes occur as osteoclasts directly resorb foreign material or macrophages phagocytose it.[5] Fine differences in material composition may significantly affect these processes and the rate at which they occur.

It is noteworthy that, if resorption occurs too rapidly, an overly exuberant inflammatory response may be observed. Depending on the material, particle size

and environment, an overly intense inflammatory reaction may produce a foreign body reaction and limit bone regrowth.

Other resorbable bioceramics have been developed with differing resorption properties. For example, beta Tricalcium Phosphate (ß-TCP) and Calcium Sulphate (CS) have resorption rates of approximately six months and three weeks, respectively. The intermediate resorption rate of ß-TCP is seen as an advantage in some applications, but it is not as strong as HA.[4,6] The short resorption time of CS is too rapid to support bone ingrowth in most applications but it has been considered as a drug carrier providing release of incorporated products.[7,8]

c. Relevant terms

i. Biphasic calcium phosphates (BCPs)

BCP refers to mixtures of different constituent bioceramics. In order to balance structural and resorption properties, 'biphasic' materials have been developed with a mixture of different components.[3,4,6,9,10–12] For example, HA has been mixed with ß-TCP (e.g., BoneSave™ Bone Void Filler by Stryker is a 20% HA/80% ß-TCP mixture).

ii. Phase purity

Phase purity refers to the relative mass percentages of different crystalline phases within a bioceramic. This is a recognized, but not well understood, variable affecting the biological integration of hydroxyapatite-based biomaterials. Minor amounts of specific impurities, such as calcium oxide (CaO) and tricalcium phosphate (TCP), may be present either as deliberate additions, such as in biphasic materials, or as a result of decomposition during sintering,[13] which is a high heat process used in manufacturing to consolidate ceramic particles into a cohesive mass.

Research shows that phase purity of bioceramics such as HA is an important factor in the establishment of an ideal environment for bone growth. In fact, reduction in the phase purity of HA can reduce bone apposition,[14]

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accounting for some of the variable clinical outcomes observed with different bone graft materials. For example, a comparison of phase pure HA (Ca:P ratio 1.67) with a material containing around 5% CaO (Ca:P ratio 1.70) implanted into rabbit femurs showed a good degree of direct bone apposition in the phase pure samples, but not in the less phase-pure retrieved samples.[15]

iii. Porosity and interconnectivity

In order to manufacture a synthetic bone scaffold which simulates natural bone, one must consider the architecture and geometry that gives bone its strength, yet allows nutrients and metabolic byproducts to traverse its structure. Terms such as macroporosity, microporosity, and interconnectivity have been devised. Macroporosity refers to the relatively large sized pores within a bioceramic's architecture (i.e. pores greater than 50 µm in diameter). Microporosity refers to more fine scale differences. Interconnectivity refers to the degree to which pores are joined to each other within a porous structure, and thus can communicate with their surroundings.

Many authors have reported greater turnover for materials with increased porosity and interconnectivity.[16,17,18,19,20] This may lead to more rapid equilibration

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with the surrounding milieu and allow greater migration of cells such as macrophages, mesenchymal stem cells, osteoblasts and osteoclasts. These cellular products may attach, proliferate, and differentiate allowing for osteogenesis and angiogenesis.[18,21] Without this interconnected porosity, the osteoclasts would have to tunnel through the bone matrix to resorb the synthetic material prior to formation of new bone, which would be a much slower process. It is suggested that a porosity threshold exists around 60%, below which sustainable bone integration is difficult to obtain.[22,23]

Conversely, increasing the degree of porosity and interconnectivity too much may jeopardize mechanical stability, especially in a setting where load bearing is a key consideration. Therefore, it is suggested that appropriate modification of the macrostructure be made to distribute loads and match the intended host tissue in order to improve biocompatibility. However, in load protected grafting applications, i.e. small defect filling or those where stabilization is employed, this is not normally a limiting factor. Additionally, there should not be excessive mechanical support as this may lead to micro-environment

stress shielding which would hinder bone formation[24] since Wolff's Law would not be in operation.

III. Role of Silicon in Bone Formation

Silicon has been shown to affect bone formation. In the 1970's, Carlisle, Schwarz and Milne independently reported animal studies noting that silicon deficiency results in abnormal bone formation. This was correlated with decreased deposition of both extracellular matrix (collagen) and bone mineral (HA).[25,26] These findings have been confirmed by many studies over the past few decades evaluating the soluble silicate anion (orthosilicic acid).[25–28]

The average daily intake of silicon in the western world is about 20-50 mg/day, although it is lower in women (24 ± 12 mg/day at the age of 26–39 years) than men (37 ± 23 mg/day at the age of 26–39 years). Decreased intake has also been noted with diet changes associated with increased age. Silicon is found in cereals and grains (e.g., breakfast cereals, bread, beer), some fruits and vegetables (e.g., bananas, raisins, beans, lentils), and unfiltered drinking water.[29]

The complete role of silicon in human bone metabolism has not been fully characterized. Typical silicate plasma concentrations after ingestion of silicon-containing foods stimulate human osteoblasts and osteoblast-like cells to secrete type I collagen and other biochemical markers of bone cell maturation and bone formation.[27] Soluble silicate may stabilize aqueous hydroxyl-radical species,[30] and some have suggested silicate involvement in the radical-dependent prolyl-hydroxylase pathway[31] during type I formation. Others have suggested a structural role in the cross-linking and stabilization of collagen and glycosaminoglycans.[32]

Silicon has been found in greatest concentrations in immature bone.[33] In this setting, silicon and calcium contents rise congruently in osteoid tissue. In the more advanced stages of mineralization, the silicon concentration falls markedly while the calcium concentration approaches higher proportions in bone HA. It has been suggested that silicon is involved in the initiation of calcification through some effect on the preosseous matrix.[34]

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Interestingly, food preparations in the western world have reduced the silicon availability in diets due to filtration and treatment of drinking water, processing of cereals, and also hydroponic growth of vegetables.[35] The clinical implication of this has not yet been clearly demonstrated.

a. Preclinical studies

Silicon-deficient diets have been shown to result in bone malformations, decreased acid and alkaline phosphatase activities, and decreased collagen and copper concentrations in animal trials. Copper is known to be involved in collagen cross-linking in bone, which explains the mechanism by which silicon affects bone formation. This is supported by studies showing that rats fed silicon-deficient diets (less than 2.0 g kg^{-1} diet) demonstrated decreased acid and alkaline phosphatase (osteoclast and osteoblast markers) activities in their femurs compared to rats fed adequate amounts of silicon (35.0 g kg^{-1}diet).[36,37] Silicon-deficient rats also had decreased plasma ornithine aminotransferase activity, a key enzyme involved in collagen synthesis.[38] The authors suggest that decreased plasma silicon and increased plasma alkaline phosphatase levels measured in silicon-inadequate rats indicate that bone mineralization proceeded at a slower rate in that group. Silicon-adequate rats exhibited the better bone mineralization.[39]

Reduced levels of copper, manganese and zinc, all minerals observed to be critical to bone mineralization in horses, were also noted in the femur and vertebrae of silicon-deficient animals. The decrease in femoral calcium and vertebral phosphorus observed in silicon-deficient rats was prevented by supplementing the diet with 35 mg kg^{-1} of silicon, providing some evidence to suggest that physiological intake of silicon can alleviate silicon deprivation. Conversely, silicon supplementation has been shown to inhibit osteopenia induced by ovarectomy in rats. Several trials noted that excess silicon was harmlessly excreted.[28,40]

Using electron probe microscopy, Carlisle showed that silicon (up to 0.5%) was present in active calcification sites in young mouse and rat bone.[33] Chicks fed a diet optimized for growth but silicon-deficient exhibited skull abnormalities associated with reduced collagen content. Silicon deficiency was incompatible with normal growth and skeletal development in the chick, but the abnormalities were corrected by a subsequent silicon supplementation.

b. Human studies

Jugdaohsingh, et al. performed a clinical study evaluating the role of dietary silicon. In a cross sectional, population-based study of 2,847 participants, a positive association

was found between dietary silicon intake and bone mineral density (BMD). Increased BMD was found at the hip in men and premenopausal women, but not in postmenopausal women.[41]

In another study of 53 osteoporotic women, silicon supplementation was associated with a significant increase in the BMD of the femur.[42] The positive results of these studies suggest that silicon supplementation, along with calcium and vitamin D, may be useful in the fight against osteoporosis.

Based on all the current research, silicon is now considered a critical nutrient to better manage the effects of age on the body. Increased silicon may be obtained through increased intake of foods rich in silicon, taking plant extracts or by using supplements, as discussed earlier.

IV. Silicate-substituted Calcium Phosphate

Actifuse™ Synthetic Bone Graft is a product developed by ApaTech Ltd. (London, UK) which is a phase-pure 80% porous calcium phosphate material in which a portion of the phosphate (PO4) groups are selectively replaced with silicate ions (SiO4). This is done in a proportion that approximates the (0.8 wt%) silicon concentration in immature bone. As a result, a novel silicate-substituted calcium phosphate material is created, distinctly different from traditional tricalcium phosphates and hydroxyapatites. Consistent macro- and microporosities and a high degree of interconnectivity are key structural features in Actifuse and have been proven to facilitate osteogenesis and angiogenesis while avoiding mechanical discontinuities.[43] In the United States, Actifuse has been cleared to market as a bone void filler. It is intended for orthopedic applications as a filler for gaps and voids that are not intrinsic to the stability of the bony structure. The product provides a bone void filler that resorbs and is replaced by bone during the healing process.

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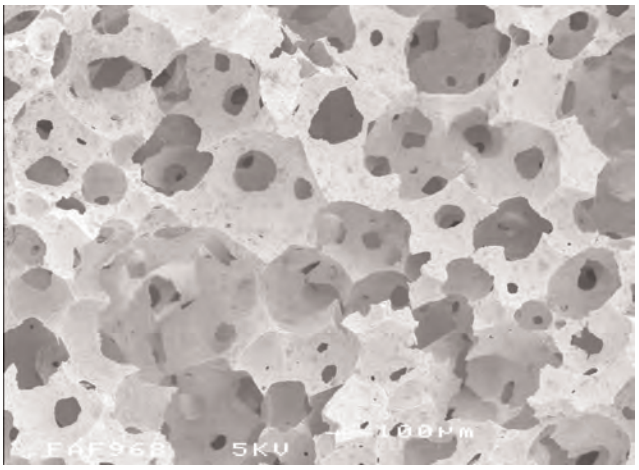
Preclinical studies have suggested that the bioactivity of HA is significantly enhanced by the incorporation of silicate ions into its lattice. Dense, slotted cylinders composed of stoichiometric hydroxyapatite and 1.2 wt% silicate substituted hydroxyapatite (S-HA) were implanted in the distal end of the femur of 6 month old New Zealand white rabbits for a period of 3 weeks. Results showed greater penetration of bone within the slots in the silicate group.[44]

When compared to Vitoss® (Orthovita, Malvern, PA), a resorbable beta tricalcium phosphate scaffold that is highly porous, Actifuse showed increased bone

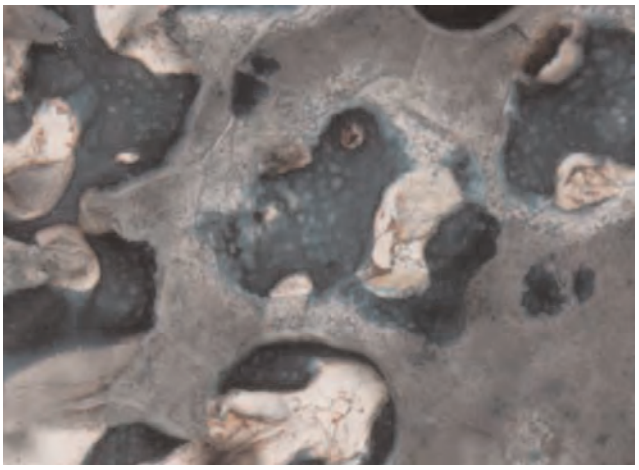
Figure 2

A. Scanning electron microscope image of Actifuse Synthetic Bone Graft.

B. Histological slide of Actifuse following 3 weeks implantation in a cancellous bone defect in the rabbit knee (scale bar indicates 100 microns). Goldner's Trichrome stains Actifuse in grey, bone in green-black, and collagen in red.



A.



B.

growth and earlier progression to mature bone in a lapine study with distal femoral cancellous defects. Both products were compared at different time intervals of 1, 3, 6

When compared to Vitoss®, Actifuse showed increased bone growth and earlier progression to mature bone in a lapine study with distal femoral cancellous defects.

and 12 weeks. Results showed evidence of bone formation with Actifuse at earlier time intervals with higher levels of organized and mineralized bone and marrow when compared to Vitoss, which exhibited extensive scaffold degradation and fibrous tissue formation.[45]

Actifuse also showed equivalent results to autograft spine fusion rates in a study involving 18 sheep. Instrumented posterolateral spine fusions were performed and evaluated. Actifuse provided an osteoconductive matrix for bone infiltration, whereas autograft provided both an osteoconductive scaffold as well as osteogenic stimuli, yet Actifuse was able to overcome the biological disadvantage and produce a robust fusion callus with osteoblast and osteoclast activity equivalent to autograft-supplemented fusion tissue at 2 and 6 months post-op. However, autograft activity levels decreased from 2 to 6 months while activity in Actifuse remained constant. Additionally, the silicate substitution in the calcium

Actifuse had a substantial increase in graft volume compared to autograft, and also showed evidence of pseudo cortex formation around the fusion mass.

phosphate matrix enhances the bioactivity of the material, promoting osteoblast migration and spontaneous formation of bone spicules within the matrix not associated with creeping substitution. This spontaneous bone formation has been previously noted with other silica-containing graft materials.[46,47] Actifuse had a substantial increase in graft volume compared to autograft, and also showed evidence of pseudo cortex formation around the fusion mass.[48]

How much silicate is required to maintain or even stimulate bone formation remains to be defined. However, studies have shown that there is an optimal amount of silicate that enhances local bone bioactivity, and this is supported by the finding that a crystalline silicate-substituted porous calcium phosphate containing 0.8 wt% silicon substituted as silicate showed higher in vivo bioactivity than similar materials containing 1.6% or 0.4 wt% silicon.[49] Both silicate-substituted materials had a higher in-vitro bioactivity than silicate-free materials.[50]

Additionally, other authors have proposed that the increased solubility conferred by silicate substitution in calcium phosphates drives biological apatite precipitation and that increasing the amount of silicon substituted increases the rate of bone apposition.[51–53] It is also important to point out that small amounts of silicate appeared to promote rapid apposition of immature bone, while increasing the amount of silicon to 0.8 wt% appeared to restore/promote the apposition of more mature lamellar bone at earlier time points.[53,54] This supports the use of similar amounts of silicate in products such as Actifuse in order to maximize osteointegration at the graft site. This level of silicate substitution also allows the retention of phase purity, whereas substitutions above that level resulted in phase separation into materials comprising calcium phosphate and silicon-substituted calcium phosphate phases at 1100°C.[55]

V. Conclusion

It is evident that the development of new bone graft substitutes in the orthopedics arena is becoming more focused on harnessing patient's inherent osteoconductive, osteoinductive and osteogenic properties. Simultaneously, surgeons are changing their operating procedures to try to avoid the potential morbidity associated with autograft harvest, particularly from the iliac crest. Porous ceramic materials are one potential class of bone graft material. Maximizing structural and chemical properties are important objectives.

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