MONOGRAPHY

BONE and SILICIUM (Silicon).
literature review

Courcelles – February 4, 2020

1. INTRODUCTION

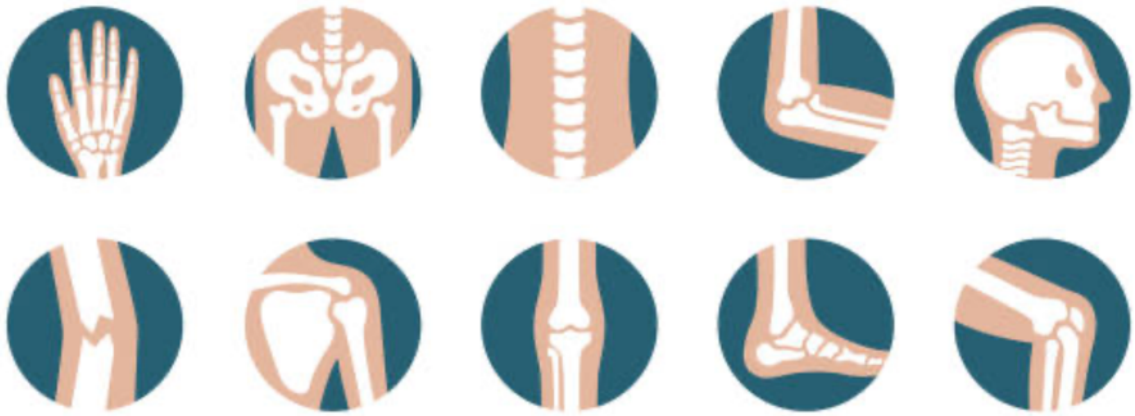
Bone is a unique tissue in that unlike any other tissue type in the body it can regenerate without leaving a fibrous scar. However, when a bone defect is too large, for example as a result of bone tumor removal or trauma, it is a critical size defect, which the body can no longer heal. It has been estimated that the number of these cases is up to half a million each year in the US alone, and usage of specific vitamins and minerals supplements have been suggested as one course of therapy to address this issue.

In order to function as a successful food supplement, the mineral has to promote the healing capabilities of bone tissue. Bone is a dynamic tissue that is constantly remodeled in the body by osteoclasts that resorb bone and osteoblasts that synthesize new bone extracellular matrix and mineralize it. To promote the formation of new bone, a food supplement must not only activate osteoblasts to make new bone but also stimulate the collagen type 1 synthesis to remodel it into functional, load bearing bone.

Silicon based food supplements such as ortho-silicic acid is known for his beneficial effects on bone healing. Although the exact mechanism is not completely understood, Ingestion of bioavailable silicium increase the proliferation and differentiation of osteoblasts, stimulates collagen type I synthesis and participate to the reticulation of Glycosaminoglycan and collagen.

The differentiation of osteoblasts is vital for the regeneration of all mesenchymal tissues including bone. Ingestion of bioavailable silicon have also been reported to support the differentiation of osteoclasts. For these reasons silicium based food supplements have been suggested to be used as supplements in bone repair.

In this monography the effects of silicium on bone metabolism were examined.



REVIEW OF LITERATURE ABOUT BONE COMPOSITION AND BONE METABOLISM

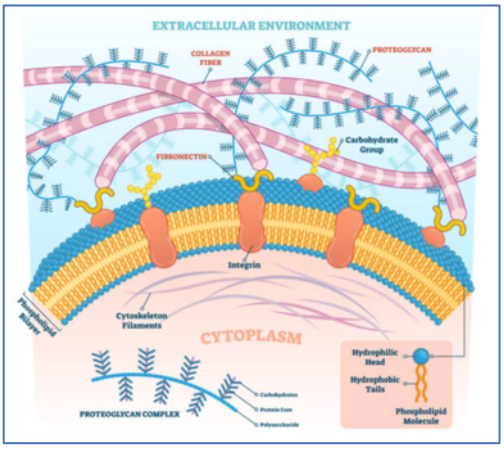
extract of Timothy Wilson, 2011

1. ***Bone***

Bone is a complex and dynamic living tissue that engages in continuous dynamic remodeling—breaking down old bone and replacing it with new bone. It functions as mechanical support and assistance for movement in vertebrates, and as protection for many internal organs against injury, the brain for example. In addition to these mechanical qualities, bone also serves as the organ of blood cell production, i.e. hematopoiesis, and according to newest studies also as the source of bone marrow stromal cells (BMSCs), which can differentiate into mesenchymal lineages and repair injuries, for example in bone or cartilage (Caplan 1991). Bone also functions in mineral homeostasis and stores several vital minerals, especially calcium and phosphorus, which it can release into the bloodstream to maintain critical mineral balances.

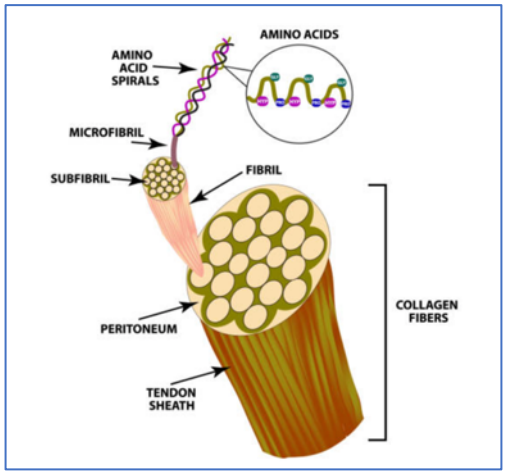
2. ***Extracellular matrix***

Bone tissue consists of cells and extracellular matrix (ECM) like all connective tissues, and in bone the amount of ECM is especially abundant. The ECM is composed on an organic phase of collagens and other proteins, which provide elasticity and tensile strength, and an inorganic phase of minerals, predominantly calcium and phosphate, which give bone hardness and load-bearing capabilities. The organic matrix accounts for 20-40% of bone mass of which 95% is composed of structural collagens. The remaining part consists of proteoglycans and other non-collagenous proteins. The inorganic phase accounts for 50-70% of bone mass and cells a mere 1%. The rest is water, around 5-10%, and lipids, 3% (Robey and Boskey 2008).



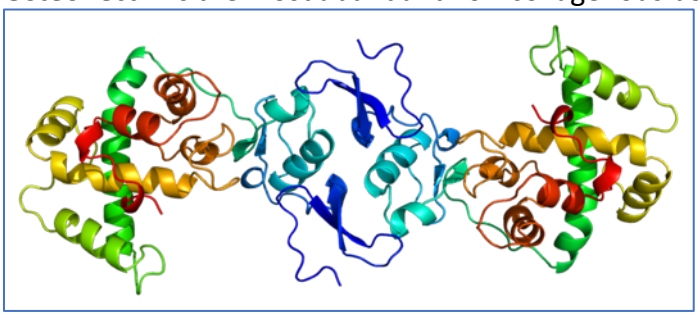
2.1. Organic phase

Type I collagen is the most abundant and important protein of the organic phase of bone. This triple helical protein composed of two α1 chains (also called COL1A1) and one α2 chain (COL2A1) comprises 85-90% of the organic matrix. Collagens are. rope like proteins that have a unique repeating polypeptide structure of $(Gly\text{-}X\text{-}Y)_n$ where X and Y often represent proline and hydroxyproline, respectively. Once outside the cell the triplex undergoes covalent cross-linking within and between other collagen molecules to form fibrils. Type I collagen gives bone tensile strength, and also contains peptide motifs which are integrin binding sites for osteoblasts (McCann et al. 1997), and a

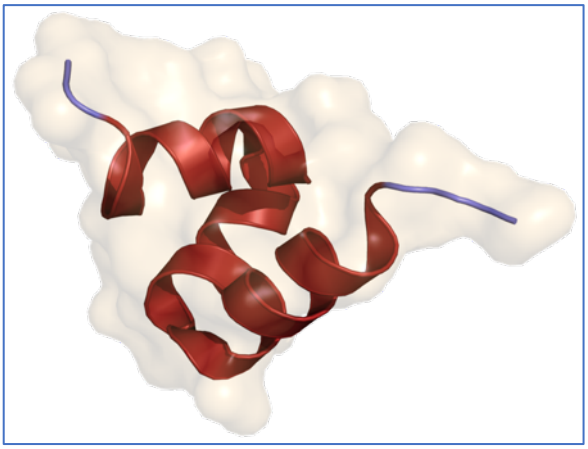


structural template for mineral deposition (Landis et al. 1996; Landis 1999). The vitality of type I collagen for healthy bone formation is demonstrated by many single base mutations that disrupt the assembly of collagens and lead to bone defects commonly lumped together as the genetic disease osteogenesis imperfecta (Pace et al. 2002; Primorac et al. 2001). There are also trace amounts of type III, V, XI and XIII in bone (Gehron Robey 1989; Gentili and Cancedda 2009). Type III collagen has been detected in developing and remodeling bone (Keene et al. 1993) and in blood vessels and the bone marrow during fracture healing (Hiltunen et al. 1993), and type V collagen has been shown to have a role in the diameter control of type I collagen fibrils (Wenstrup et al. 2004).

Osteonectin is the most abundant non-collagenous bone ECM protein. It is a glycoprotein that has multiple collagen Ca2+ binding sites and has been shown to be a potential nucleator of hydroxyapatite (Maurer et al. 1996; Young et al. 1992). It also regulates cellular function and experiments in mice showed that a deletion of ostenectin cause osteopenia with defects in the function of both osteoblasts and osteoclasts (Delany et al. 2000).



Osteocalcin (OCN) and Matrix gla protein (MPG) are gamma-carboxyglutamic acid (Gla) containing proteins which have vitamin K dependent post-translational modifications. Osteocalcin is the second most abundant non-collagenous protein in bone ECM. It has a very narrow expression pattern being made only by osteoblasts and osteocytes, while matrix gla protein is also expressed in cartilage and arteries. They are both inhibitors of mineralization. OCN deficient mice have increased bone formation (Ducy et al. 1996) while MPG knock out mice had spontaneous calcification arteries and cartilage (Luo et al. 1997).



Biglycan and Decorin belong to the family of small leucine-rich proteoglycans. Proteoglycans are macromolecules that contain acidic polysaccharide chains, glycosaminoglycans, attached to a core protein. Both biglycan and decorin are heavily enriched in bone ECM, but their role in bone physiology is still being investigated. They influence bone cell differentiation and proliferation, and mineral deposition (Waddington et al. 2003) as well as cell behavior by blocking adhesion motifs of RGD-containing molecules and by binding TGF-β (Halpert et al. 1996, Hildebrand et al. 1994). Biglycan deficient mice develop lower peak bone mass in a phenotype resembling osteoporosis (Xu et al. 1998) that seems to be cellularly mediated by defective osteoblasts which are fewer in number and less responsive to TGF-β (Chen et al 2002). Decorin deficient mice have no gross bone phenotype, but decorin/biglycan double

knockout mice have even more defective long bones than biglycan knockouts, suggesting that decorin can partly compensate for biglycan in its absence (Bi et al. 2005; Corsi et al. 2002).

Alkaline phosphatase (ALP) is produced by osteoblasts in phospholipid membrane bound matrix vessels, and it is ubiquitous in bone, but also in liver, kidney, placental and intestinal tissues. ALP plays a direct role in the induction of hydroxyapatite deposition on ECM proteins (Anderson 1995; Beertsen and van den Bos 1992; Storrie and Stupp 2005) and aids in the hydrolyzation of organic phosphate esters producing an excess of free inorganic phosphate which initiates biomineralization (Beertsen and van den Bos 1992; Nuttelman et al. 2006). Although the mechanism of its action is not completely understood, ALP remains an excellent indicator of osteodifferentiation and mineralization.

RGD containing glycoproteins contain an arginine-glycine-aspartic acid (RDG) tripeptide which is a ligand motif for the integrin class cell-surface molecules. There are many proteins of this type in the bone matrix and they convey attachment points to cells. ***Fibronectin*** is one of the most abundant non-collagenous proteins in bone ECM and has been shown to regulate osteoblast differentiation (Moursi et al. 1996, 1997) and their subsequent survival (Globus et al 1998). It may also regulate mineralization (Couchourel et al. 1999; Daculsi et al. 1999) by binding to other matrix proteins and modifying their activities (Dallas et al. 2000; Merle et al. 1999). ***Osteopontin*** has been suggested to be required for the resorption of bone by increasing vascularization related to it. ***Bone sialoprotein*** (BSP) is involved in regulating hydroxyapatite formation in bones and teeth (Fisher et al. 2001). It promotes the initial formation of mineral crystals and is considered an early marker of osteogenic differentiation (Chen et al. 1992, 1994; Kasugai et al. 1992; Sodek et al. 1992). ***Dentin sialophosphoprotein*** (DSPP) was first believed to be tooth-specific, but was later found to be expressed also in bone (Qin et al. 2002, 2003). ***Dentin matrix protein*** (DMP) was also first identified in dentin, but later discovered in other tissues. In adult bone DMP is associated with the mineralization process (Feng et al. 2002). ***Matrix extracellular protein*** (MEPE), also called osteoblast/osteocyte factor 45 (OF45), is a highly serine/glycine rich protein which is expressed in mineralized tissues (Petersen et al. 2000). ***Thrombospondin 2*** is an abundant protein in 16 Review of Literature bone (Gokhale et al. 2001; Robey et al. 1989). It inhibits the number of bone cell precursors (Hankenson and Bornstein 2002) and thrombospondin 2 knockout mice have increased bone density and cortical thickness (Hankenson et al. 2000). ***Vitronectin*** is a multifunctional protein present in the ECM and blood, which binds glycosaminoglycans, collagen, plasminogen (Schvartz et al. 1999). In bone tissue it is known to induce osteoclast polarization (Takahashi et al. 2007).

2.2. Inorganic phase

The mineral phase of bone consists almost completely of calcium phosphate hydroxyapatite (HAp) [Ca5(PO4)3(OH)] with some impurities such as carbonate, citrate, magnesium, fluoride and strontium (Cowin 2001; Leventouri 2006). This phase gives bone its rigidity, hardness and load-bearing capabilities, and facilitates bone to act as a reservoir for calcium and phosphate and other ions. The inorganic phase deposition begins immediately after the formation of the organic phase with the formation of a primary hydroxyapatite crystals. The crystals then grow to needles of variable length and of widths of about 30 to 45 nm and thickness of approximately 5 nm, and their longest side lies parallel to the axis collagen fibers. After the primary crystal is formed, the crystal may branch into several dimensions, becoming larger and more perfect as the bone matures. Secondary mineralization also occurs when new crystals are formed on the old one. Bone mineral matrix formation is a slow process which takes several moths or even years to reach really high densities of bone (Boskey 2006, Robey and Boskey 2003).

3. Cells

Bone is constantly being built, resorbed and then rebuilt through a physiological process called remodeling that is carried out and
carefully controlled by a variety of cell types. Osteoblasts synthesize and mineralize bone matrix, which is maintained by osteocytes, and as required, resorbed by osteoclasts.

3.1. Osteoblasts

Osteoblasts are cells of mesenchymal origin that are nearly indistinguishable from fibroblasts, except for a mineralized ECM, and the expression of certain bone specific genes, such as osteocalcin and the transcription factors Runx2 , Osterix (OSX), ATF4.

The process of bone formation in vivo can be divided into three stages. In the first stage osteoprogenitor cells proliferate and start to express type I collagen and transiently osteopontin. During the second stage osteoblasts start to form bone matrix by synthesizing and secreting type I collagen. In the third stage mineralization begins as the osteoblasts start expressing osteocalcin, osteopontin and collagenase. The active matrix producing osteoblasts have a large nucleus, enlarged Golgi apparatus and extensive endoplasmic reticulum, which is typical for a cell actively engaged in secretion. Active osteoblasts are also highly enriched in ALP and secrete type I collagen and other matrix proteins (Lian et al. 2003).

3.2. Osteocytes

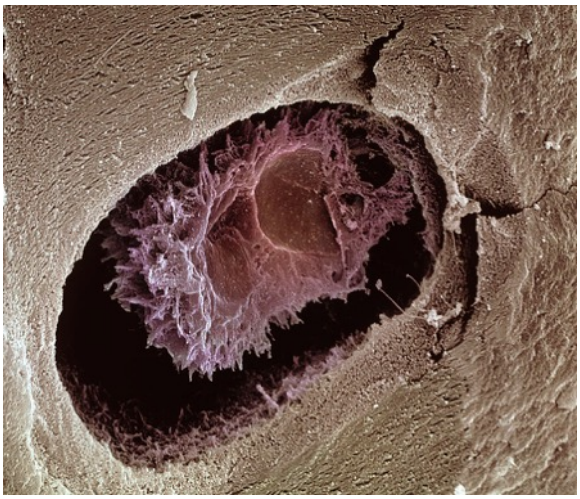
Osteocytes are terminally differentiated osteoblasts, which have been incorporated into newly composed bone matrix. It has been estimated that around 10-20% of osteoblasts differentiate this way into osteocytes (Aubin and Turksen 1996). They lie within lacunae within mineralized bone and have extensive filipodial processes that lie within canaliculi in bone tissue (Bonewald 1999). Osteocytes maintain connection with each other and bone lining cells on the bone surface through multiple filipodial cellular processes that are created before and

during matrix synthesis (Palumbo 1986). The transformation of a motile osteoblast into an entrapped osteocyte takes about three days, and the lifespan of mature osteocytes in bone that is not turned over can be decades (Frost 1963; Marotti et al. 1990).

The function of osteocytes is still relatively poorly known, but they are the most abundant cells in bone and they are actively involved in maintaining the bone matrix, and osteocyte death is eventually followed by matrix resorption (Junqueira et al. 1995). It has been suggested that osteocytes transmit mechanical signals within bone. They make up a synctial connection with osteoblast and bone lining cells which is capable of mechanosensation (Aarden et al. 1994; Burger and Klein-Nulend 1995). Transmission of mechanical signals to the osteocyte cytoskeleton through cell surface receptors occurs both directly through direct contact with the solid matrix structure of bone, but also through fluid pressure and shear stresses imparted by fluids moving through the lacunocanalicular system due to flow load-induced flow (Knothe Tate 2003). Osteocytes have also been suggested to regulate mineral homeostasis through a process called osteocytic osteolysis (Noble 2008).

3.3. Osteoclasts

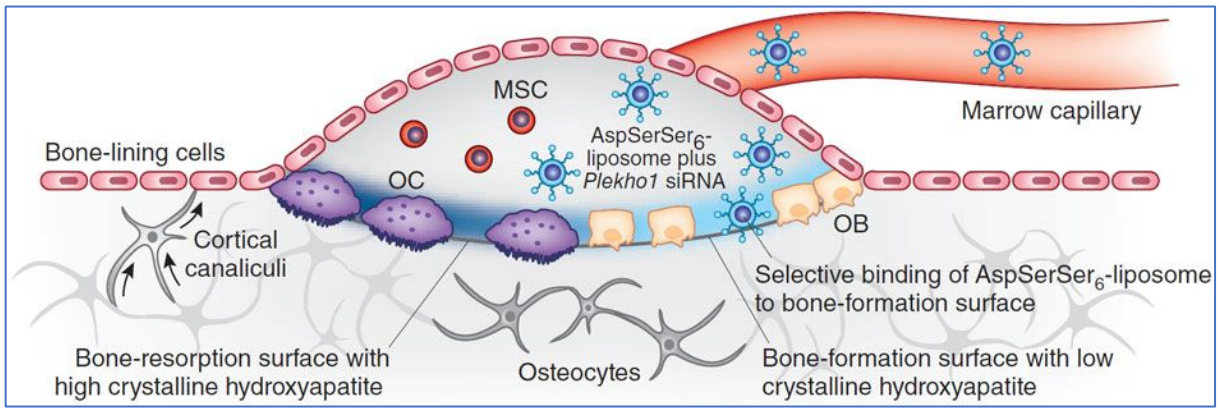
Osteoclasts are multinucleated cells that are specialized in the removal of bone tissue. They derive from hematopoietic stem cells and progress through the colony forming unit for granulocytes and macrophages (CFU-GM) and the CFU for macrophages (CFU-M) to the preosteoclast and finally to the mature multinucleated osteoclast (Teitelbaum and Ross 2003; Greenfield and Rubin 2005). Osteoclasts are only found in bone tissue where they differentiate with the help of stromal cells (Takahashi et al. 1988). Close contact with stromal cells in bone tissue is required for the production of two hematopoietic factors, the TNF-related cytokine RANKL and the polypeptide growth factor CSF-1 that are both necessary and sufficient for osteoclastogenesis (Lacey et al. 1998; Yasuda et al. 1998), and for the subsequent activation of RANK on the surface of hematopoietic precursor cells (Nakagawa et al. 1998; Hsu et al. 1999). These two factors are required to induce the genes that typify the osteoclats lineage, including tartrate resistant acid phosphatase (TRACP) and cathepsin K.



In order to resorb bone, the osteoclast attaches to its surface and begins to assume a polarized morphology. An actin ring that creates a tight junction between the cell and the underlying bone forms and the osteoclast's cell membrane inside this sealing zone becomes ruffled (Lakkakorpi et al. 1989). This vacuole is then acidified by the export of hydrogen ions by the ATP6i complex (Li et al. 1999), which dissolves the solid hydroxyapatite. The organic phase is then degraded mainly by the secretion of cathepsin K (Gowen et al. 1999), although other

proteinases, such cathepsin D, B and L (Drake et al. 1996) and matrix metalloproteinases (Everts et al. 2006), are also present. TRACP is also expressed strongly during osteoclast differentiation and is used as a cellular marker for osteoclasts. TRACP functions as an acid phosphatase and it is capable of generating reacting oxygen species, which have been shown to facilitate collagen degradation, and it may thus be important in the final degradation of resorption products (Väänänen and Zhao 2008). After matrix degradation the degradation products are removed from the resorption lacuna by transcytosis from the ruffled border to a functional secretory domain, where they are liberated into the extracellular space (Nesbitt and Horton 1997; Salo et al. 1997).

3.4. Bone lining cells



Bone lining cells are thin, elongated cells that cover the surface of bone when it is not under remodeling. The retraction of these cells from the bone surface is a mandatory step before osteoclastic bone resorption (Zambonin-Zallone et al. 1984). It has been proposed that bone lining cells are a subpopulation of osteoblasts, as bone lining cells, like osteoblasts, can enwrap and resorb collagen (Everts et al. 2002), which predisposes bone to resorption by increasing mineral exposure (Chambers and Fuller 1985). It has also been proposed that bone lining cells clean osteoclast resorption pits after matrix degradation and deposits deposit a thin line of collagen to form a cement line which demarcates sites of new bone formation, giving them a role in the regulation of bone remodeling (Everts et al. 2002).

Bones Composition

Extracellular Matrix		Cells
Organic Matrix	**Inorganic**	
90%, • type I collagen 10% • other types of collagen, • non-collagenic proteins, (osteocalcin, Osteonectin, osteopontin, alkaline phosphatase, ProlineHydroxylase,...) • lipids + other macromolecules. • Proteoglycan • glycoproteins	Calcium hydroxyapatite $[CA_{10}(PO_4)_6(OH)_2]$ K, Sr, F, Si	• Osteoblasts, • Osteocytes, • Osteoclasts • Bone lining cells

4. ***Remodeling***

Bone undergoes continual growth, modeling and remodeling during life. Modeling is a process by which bones change their overall shape in response to physiological or mechanical forces, leading to a gradual adjustment of the skeleton to the forces it encounters. This process is described by Wolff's law, according to Wolff who in 1892 discovered the skeleton's ability to adapt to changes (Wolff 1892). Bone modeling is less frequent in healthy adults than bone remodeling (Kobayashi et al. 2003). Modeling differs from remodeling in that during modeling bone formation is not as tightly coupled to resorption as in remodeling. Bone remodeling is the process by which bone is constantly renewed to repair accrued microdamage and to maintain bone strength and to regulate mineral homeostasis. During remodeling small areas of bones tissue are removed and then replaced by new matrix, which is subsequently mineralized. It has been estimated that approximately 10-15% of bone surface is at any time undergoing remodeling (Kanis et al. 1995). The remodeling process advances in a cycle with four distinct phases: activation, resorption, reversal and formation and mineralization (Figure 2). The time when there is no remodeling going on is called the resting phase.

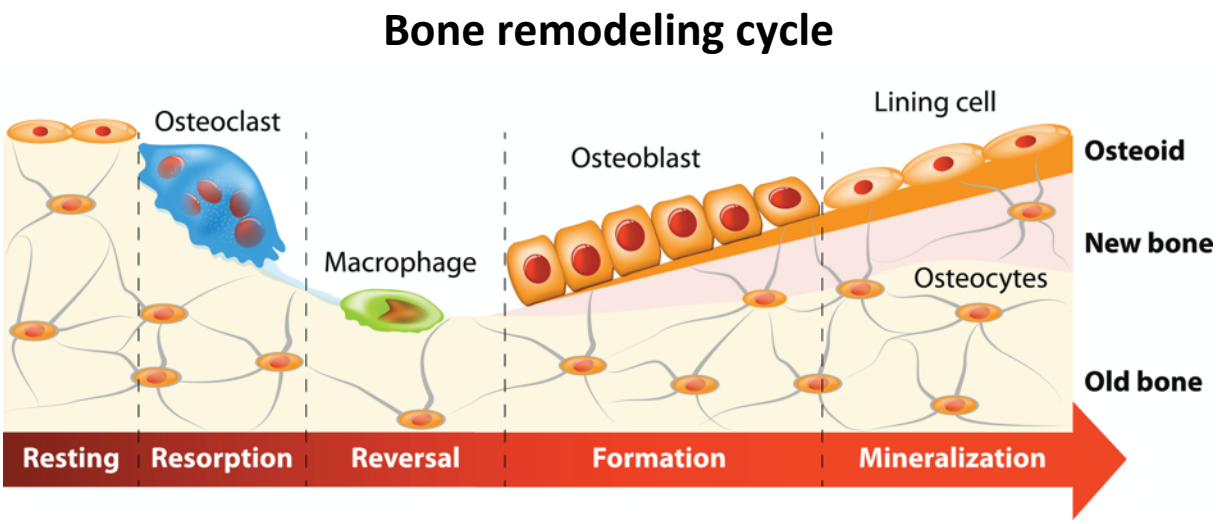


Figure 2. Bone remodeling cycle. Old bone is resorbed, and new bone is formed in a tightly controlled sequence of events.

During the activation phase mononuclear monocyte-macrophage osteoclast precursors are activated and recruited form the circulation (Roodman 1999). Bone lining cells retract from the surface (Zambonin-Zallone et al. 1984) and multiple mononuclear cells fuse to form multinuclear maturing osteoclasts, which then bind to the surface of the bone. The resorption phase starts when osteoclasts have attached to the bone, polarized and formed a ruffled border. Bone resorption has been detailed earlier (see 2.1.3.3 Osteoclasts). This phase takes about two to four weeks to complete, after which osteoclasts go into apoptosis and bone resorption is terminated (Eriksen 1986; Reddy 2004). During the reversal phase bone resorption transitions to bone formation. Osteoblast-like cells (Mulari et al. 2004) or bone lining cells (Everts et al. 2002) appear on the surface of the resorbed bone and finalize the resorption phase and prepare the surface for osteoblasts. A cement line, a specialized matrix rich in osteopontin and other phosphoproteins is formed to demarcate new and old bone (McKee and Nanci 1996). In the bone formation and mineralization phase osteoblast

precursors proliferate and differentiate at the remodeling site and start synthesizing new collagen matrix. They also initiate matrix mineralization by releasing small matrix vehicles which contain calcium and phosphate and alkaline phosphatase (Anderson 2003). Bone formation takes approximately four to six months to complete, after which 50-70% of osteoblasts undergo apoptosis, with the rest becoming osteocytes that are buried within the matrix or bone lining cells. The new bone then enters the resting phase which can continue for years until a new remodeling cycle is initiated.

5. ***Healing***

Bone is a unique tissue, which can regenerate itself completely after injury (Sommerfeldt and Rubin 2001). The processes of bone healing are similar to the healing of other tissue types, but unlike bone, other tissue types can only heal themselves by the formation of a scar. Bone, on the other hand, after healing and remodeling will retain its structural and functional properties (Glowacki 1998). The process of bone healing follows sequential phases of hematoma formation, acute inflammation, callus formation, mineralization and remodeling (Hollinger and Wong 1996). Initially a hematoma forms on the injury site and acts as a source of platelets and hematopoietic cells, such as neutrophils, monocytes and macrophages, which initiate the inflammatory cascade by secreting growth factors such as platelet derived growth factor (PDGF), TGF-β, TNF-α and others, which have a role in chemotaxis, angiogenesis and mesenchymal cell regulation (Buckwalter et al. 1996; Gurtner et al. 2008). Callus formation begins within the first week after angiogenesis and mesenchymal cell recruitment. Mesenchymal cells differentiate into chondroblasts and start making a cartilaginous callus which bridges the site of injury. This cartilage then calcifies through a process that mimics endochondral ossification. In the remodeling phase the callus is gradually remodeled, and the pre-existing structure of the tissue is restored (Einhorn 1998).

MECHANISMS OF BONE REMODELING LINKED WITH SILICIUM (Silicon)

1. Silicon And Metabolism (Extract from Rodella L. & Al. - J Nutr Health Aging 2013, Götz & Al. Pharmaceutics - 2019)

Silicon (Si) is the second most common element in Earth's crust (28.9%), after Oxygen (45.5%). It represents the major trace element in the human body. In particular, highest Si concentrations are found in fast-growing cells such as hair, nails, bone and skin cells. The major and most important source of Si is the diet. Si daily intakes range from about 20mg/day to 50mg/day in Western countries. Higher intakes (104mg/day – 204mg/day), have been reported in China and India, where plant-based foods are the major components of the diet. **About Si absorption, the main bio-available form, for human and animals, is the Ortho-Silicic Acid (O.S.A., $SiOH_4$).**

It is well-known that Si in the form of OSA existing only in liquid, like mineral water and beer but not in foods. Nevertheless, Si is hydrolyzed to OSA at the gastrointestinal level. Among foods, highest Si levels are found in grains, especially oats, barley, white wheat flour and some rice fractions. Si is also present in the form of synthetic compounds or silicates, but they are rarely found in the diet.

Orthosilicic acid $Si(OH)_4$ is the main Si species in man. After uptake, it is gastrointestinally absorbed and transported in the blood, mainly unbound. Only small amounts form complexes with Fe or Al at a neutral pH. In the human body, Si amounts to 1–2 g, which corresponds to 0.01% of body weight, i.e., lower than Fe and Zn. The concentration in serum ranges between 24 and 31 µg/dL. **Outside the blood compartment, it is mainly bound to gycosaminoglycans. It has been speculated that serum and tissue levels of Si might be regulated by responsive elements or transporters**, since such transporters (SITs) have been identified in plants and silicified organisms, (e.g., in diatoms or sponges). Recently, a SIT named SLc34a2 was found in mammals. Water channel aquaporins are homologous to SITs in rice, and have been detected in the small intestine and renal epithelia, and also in the bones and joints of mice. Their expression seemed to be diet dependent: Under a Si-rich diet, certain aquaporins were upregulated in kidney and calvarial bones. However, how silicic acids reach their final site of deposition in the body still has to be investigated.

Si is excreted mainly renally after glomerular filtration and can be detected in the urine. It is possible that Si levels in urine could represent a parameter for Si bone metabolism, since reduced excretion could be associated with osteopenia. After uptake, most absorbed Si is excreted after4–8 h in urine. In healthy human volunteers, the ingestion of soluble Si results in the excretion of the same quantity of Si within 24 h.

Si can be found in high levels in the extracellular matrix bound to different components, especially glycosaminoglycans. The role of Si in connective tissue development and differentiation has been discussed, since Si can form complexes with polyols like hexosamines, which are components of glycosaminoglycans and mucopolysaccharides that form extracellular matrix components.

Additionally, Si plays a role as a cross-linking element in the bridging between proteoglycans and collagens. Si supplementation in the diet shows stimulatory effects on cartilage synthesis. In the connective tissues of rats, Si concentration decreases with increasing age. Probably, Si is necessary in young animals for connective tissue and bone development.

2. Silicium And Bone : (Extract from Götz & Al. Pharmaceutics - 2019)

Due to many in vitro and in vivo studies, it can stated that **Si is beneficial for bone tissue structure and function and is associated with calcium in bone metabolism**. There is increasing evidence that Si has a positive impact on bone homeostasis. In older studies, Si deprivation led to abnormal growth and growth defects, e.g., in chickens. More recently, this was based on different animal studies where Si application increased bone density and bone turnover in osteopenic ovariectomized rats, especially when the animals were fed with Ca-reduced food. In a recent study (Jugdaohsingh et al.), a positive correlation between Si concentration in serum, bone quality, and osteocalcin levels in the serum of female rats was found. For humans, **different clinical studies have shown correlations between bone health and Si levels**. The Framingham Offspring Cohort has investigated the correlation between Si supply from food and hip and lumbar vertebral bone mineral density (BMD) measured by osteodensitometry in 1251 men and 1596 women.

Positive correlations were found for men and premenopausal women. During the Aberdeen Prospective Osteoporosis Screening Study, women ages 45 to 54 were observed for bone density and biochemical markers of bone metabolism, such as the anabolic procollagen Type 1 N-terminal propeptide (PINP) and catabolic PYD/DPD measured in serum and urine, respectively, and these factors were correlated with Si intake. In the group with the lowest Si intake, mean bone hip density was significantly lower than in the group with the highest intake. Si intake was negatively correlated with PY/DPD, indicating bone resorption, and was positively correlated with PINP, indicating bone apposition. However, these associations were not found in women with postmenopausal estrogen deficiency. Spector et al. finally investigated the effects of silica substitution in different concentrations given in addition to calcium–vitamin D supplementation in 134 women with osteopenia for 12 months and measured the N-terminal propeptide of procollagen type I as an indicator for anabolic effects. Collagen synthesis was higher in women taking 6 and 12 mg of silica after 6 and 12 months, although no differences could be obtained for bone density measurements. These **studies show that there is a correlation between the effects of Si on bone metabolisms and estrogen as well as on collagen metabolism. Taken together, it can be proposed that Si levels are associated with BMD, bone mechanics**, and probably estrogen status. In rat bones, the highest Si concentrations have been found in the low-mineralized bone of younger animals. An equal distribution in the mineral and collagen fractions of bone was shown, especially in the early stages of mineralization, which is also indicative of a close association between collagen and Si. With increasing age, total bone Si content increased. Physiologically, hydroxyapatite can be substituted by other ions, including Si. Biological apatites can contain small levels of impurities, including Si.

Silicon can promote bone formation. In MG63 osteoblasts, Si stimulation has led to cell proliferation and remarkably enhanced gene expression of collagen type I, the effects of which

were theorized to be induced by induction of the extracellular signal–regulated kinases (ERK) pathway. A conditioned medium supplemented with orthosilicic acid increased the secretion of collagen type I, alkaline phosphatase, and osteocalcin Increased expressions of different bone and osteogenesis genes in mice and men, such as bone morphogenetic protein-2 (BMP-2), collagen type I, and runx-2, have also been found Si is involved in the early stages of biomineralization, with high levels present during early calcification processes. Probably, $Si(OH)_4$ is able to induce the precipitation of hydroxyapatite (HA) from electrolyte solutions. In vitro, Si-based components can cause an alteration of the expression of genes for amelogenin, ameloblastin, and enamelin in human osteoblats-like SaOS-2 cells, which are structural components of tooth enamelum. Si inhibits the activity of macrophages and osteoclasts and stimulates osteoprotegerin (OPG) in osteoblast-like cells, counteracting the catabolic effects of receptor activator of nuclear factor kB ligand (RANKL), which is involved in the activation of osteoclasts. In the context of crosstalk between osteoblasts and osteoclasts, Si is thought to be inhibitory for the differentiation and activation of osteoclasts.

Summary about some mechanisms of bone remodeling linked with Silicium

PROMOTES

- **osteoblast-specific genes transcription,**
  Dong M, (2016) Biological silicon stimulates collagen type 1 and osteocalcin synthesis in human osteoblast-like cells through the BMP-2/Smad/RUNX2 signaling pathway. Biol Trace Elem Res 173:306–315
- **alkaline phosphatase (ALP) production,**
  Reffitt 2003, Bone 32 : 127-135
  Dong 2016, Biol Trace Elem Res 173:306-315
- **osteogenesis (by antagonizing NF-κB activation via miR-146a)**
  Zhou 2016 Acta Biomateriala 39: 192-202

DECREASES

- **activation of NF-κB (TNF-induced pathway that induces inhibition of osteoblastic activity)**
  Zhou et al., 2016, Acta Biomateriala 39: 192-202 (id 2200)
  Activity of macrophages and osteoclasts

INCREASES

- **C-telopeptide cross-linked type I collagen (CTX)**
  Geusens 2017 BMC Musculoskelet disord 18:2
- **Procollagen type I N-terminal propeptide (PINP)**
  Spector 2008 BMC Musculoskelet disord 9 :85
- **Expression of Osteoprotegerin (OPG)**
  Wiens 2010 2017 Biomaterials 31 :7716-7725

***IS USED** :*

- **Like a cross-linking agent into collagen, polyuronides and glycosaminoglicans.**
  Schwarz 1973 PNAS 70 :1608

- **To Stimulates Osteoblastic differentiation**
  Dong 2016 Biol Trace Elem Res 173:306–315
- **In Type I collagen synthesis**
  Arumugam 2004 Key Engineering Materials 254 :869

MODULATES :

- **The cross-talk between osteoblasts (SaOS-2) and osteoclasts (RAW 264.7 cells)**
  Schröder HC - J Cell Biochem. 2012 Oct;113(10):3197-206

Bone. 2003 Feb;32(2):127-35. **Orthosilicic acid stimulates collagen type 1 synthesis and osteoblastic differentiation in humanosteoblast-like cells in vitro.**

Reffitt DM[1], Ogston N, Jugdaohsingh R, Cheung HF, Evans BA, Thompson RP, Powell JJ, Hampson GN.

Abstract

Silicon deficiency in animals leads to bone defects. This element may therefore play an important role in bone metabolism. Silicon is absorbed from the diet as orthosilicic acid and concentrations in plasma are 5-20 microM. The in vitro effects of orthosilicic acid (0-50 microM) on collagen type 1 synthesis was investigated using the human osteosarcoma cell line (MG-63), primary osteoblast-like cellsderived from human bone marrow stromal cells, and an immortalized human early osteoblastic cell line (HCC1). Collagen type 1 mRNA expression and prolyl hydroxylase activity were also determined in the MG-63 cells. Alkaline phosphatase and osteocalcin (osteoblastic differentiation) were assessed both at the protein and the mRNA level in MG-63 cells treated with orthosilicic acid. Collagen type 1synthesis increased in all treated cells at orthosilicic acid concentrations of 10 and 20 microM, although the effects were more marked in the clonal cell lines (MG-63, HCCl 1.75- and 1.8-fold, respectively, $P < 0.001$, compared to 1.45-fold in the primary cell lines). Treatment at 50 microM resulted in a smaller increase in collagen type 1 synthesis (MG-63 1.45-fold, $P = 0.004$). The effect of orthosilicic acid was abolished in the presence of prolyl hydroxylase inhibitors. No change in collagen type 1 mRNA level was seen in treated MG-63 cells. Alkaline phosphatase activity and osteocalcin were significantly increased (1.5, 1.2-fold at concentrations of 10 and 20 microM, respectively, $P < 0.05$). Gene expression of alkaline phosphatase and osteocalcin also increased significantly following treatment. In conclusion, orthosilicic acid at physiological concentrations stimulates collagen type 1 synthesis in human osteoblast-like cells and enhances osteoblastic differentiation.

J Bone Miner Res. 2004 Feb;19(2):297-307. **Dietary silicon intake is positively associated with bone mineral density in men and premenopausal women of the Framingham Offspring cohort.**

Jugdaohsingh R[1], Tucker KL, Qiao N, Cupples LA, Kiel DP, Powell JJ.

Abstract

The role of dietary silicon in bone health in humans is not known. In a cross-sectional, population-based study (2847 participants), associations between dietary silicon intake and BMD were investigated. Dietary silicon correlated positively and significantly with BMD at all

hip sites in men and premenopausal women, but not in postmenopausal women, suggesting that increased silicon intake is associated with increased cortical BMD in these populations.

INTRODUCTION:
Osteoporosis is a burgeoning health and economic issue. Agents that promote bone formation are widely sought. Animal and cellular data suggest that the orthosilicate anion (i.e., dietary silicon) is involved in bone formation. The intake of silicon (Si, approximately 30 mg/day) is among the highest for trace elements in humans, but its contribution to bone health is not known.

MATERIALS AND METHODS:
In a cross-sectional, population-based study, we examined the association between silicon intake and bone mineral density (BMD) in 1251 men and 1596 pre- and postmenopausal women in the Framingham Offspring cohort (age, 30-87 years) at four hip sites and lumbar spine, adjusting for all potential confounding factors known to influence BMD and nutrient intake.

RESULTS:
Silicon intake correlated positively with adjusted BMD at four hip sites in men and premenopausal women, but not in postmenopausal women. No significant association was observed at the lumbar spine in any group. Categorical analysis by Si intake, or energy-adjusted Si intake, supported these findings, and showed large differences in BMD (up to 10%) between the highest (> 40 mg Si/day) and lowest (< 14 mg Si/day) quintiles of silicon intake. A significant association at the lumbar spine in men was also observed. Further analyses indicated that some of the effects seen for moderate consumption of alcoholic beverages on BMD might be attributed to Si intake.

CONCLUSIONS:
These findings suggest that higher dietary silicon intake in men and younger women may have salutary effects on skeletal health, especially cortical bone health, that has not been previously recognized. Confirmation of these results is being sought in a longitudinal study and by assessment of the influence of silicon intake on bone markers in this cohort.

J Nutr Health Aging. 2007 Mar-Apr;11(2):99-110. **Silicon and bone health.**
Jugdaohsingh R[1].

Abstract

Low bone mass (osteoporosis) is a silent epidemic of the 21st century, which presently in the UK results in over 200,000 fractures annually at a cost of over one billion pounds. Figures are set to increase worldwide. Understanding the factors which affect bone metabolism is thus of primary importance in order to establish preventative measures or treatments for this condition. Nutrition is an important determinant of bone health, but the effects of the individual nutrients and minerals, other than calcium, is little understood. Accumulating evidence over the last 30 years strongly suggest that dietary silicon is beneficial to bone and connective tissue health and we recently reported strong positive associations between dietary Si intake and bone mineral density in US and UK cohorts. The exact biological role(s) of silicon in bone health is still not clear, although a number of possible mechanisms have been suggested, including the synthesis of collagen and/or its stabilization, and matrix

mineralization. This review gives an overview of this naturally occurring dietary element, its metabolism and the evidence of its potential role in bone health.

Nutr J. 2010 Oct 14;9:44. **Absorption of silicon from artesian aquifer water and its impact on bone health in postmenopausal women: a 12 week pilot study.**

Li Z[1], Karp H, Zerlin A, Lee TY, Carpenter C, Heber D.

Abstract

BACKGROUND:

Decreased bone mineral density and osteoporosis in postmenopausal women represents a growing source of physical limitations and financial concerns in our aging population. While appropriate medical treatments such as bisphosphonate drugs and hormone replacement therapy exist, they are associated with serious side effects such as osteonecrosis of the jaw or increased cardiovascular risk. In addition to calcium and vitamin D supplementation, previous studies have demonstrated a beneficial effect of dietary silicon on bone health. This study evaluated the absorption of silicon from bottled artesian aquifer water and its effect on markers of bone metabolism.

METHODS:

Seventeen postmenopausal women with low bone mass, but without osteopenia or osteoporosis as determined by dual x-ray absorptiometry (DEXA) were randomized to drink one liter daily of either purified water of low-silicon content (PW) or silicon-rich artesian aquifer water (SW) (86 mg/L silica) for 12 weeks. Urinary silicon and serum markers of bone metabolism were measured at baseline and after 12 weeks and analyzed with two-sided t-tests with $p < 0.05$ defined as significant.

RESULTS:

The urinary silicon level increased significantly from 0.016 ± 0.010 mg/mg creatinine at baseline to 0.037 ± 0.014 mg/mg creatinine at week 12 in the SW group ($p = 0.003$), but there was no change for the PW group (0.010 ± 0.004 mg/mg creatinine at baseline vs. 0.009 ± 0.006 mg/mg creatinine at week 12, $p = 0.679$). The urinary silicon for the SW group was significantly higher in the silicon-rich water group compared to the purified water group ($p < 0.01$). NTx, a urinary marker of bone resorption did not change during the study and was not affected by the silicon water supplementation. No significant change was observed in the serum markers of bone formation compared to baseline measurements for either group.

CONCLUSIONS:

These findings indicate that bottled water from artesian aquifers is a safe and effective way of providing easily absorbed dietary silicon to the body. Although the silicon did not affect bone turnover markers in the short-term, the mineral's potential as an alternative prevention or treatment to drug therapy for osteoporosis warrants further longer-term investigation in the future.

J Cell Biochem. 2012 Oct;113(10):3197-206. **Silicate modulates the cross-talk between osteoblasts (SaOS-2) and osteoclasts (RAW 264.7 cells): inhibition of osteoclast growth and differentiation.**

Schröder HC1, Wang XH, Wiens M, Diehl-Seifert B, Kropf K, Schloßmacher U, Müller WE.

Abstract

It has been shown that inorganic monomeric and polymeric silica/silicate, in the presence of the biomineralization cocktail, increases the expression of osteoprotegerin (OPG) in osteogenic SaOS-2 sarcoma cells in vitro. In contrast, silicate does not affect the steady-state gene expression level of the osteoclastogenic ligand receptor activator of NF-κB ligand (RANKL). In turn it can be expected that the concentration ratio of the mediators OPG/RANKL increases in the presence of silicate. In addition, silicate enhances the growth potential of SaOS-2 cells in vitro, while it causes no effect on RAW 264.7 cells within a concentration range of 10-100 µM. Applying a co-cultivation assay system, using SaOS-2 cells and RAW 264.7 cells, it is shown that in the presence of 10 µM silicate the number of RAW 264.7 cells in general, and the number of TRAP(+) RAW 264.7 cells in particular markedly decreases. The SaOS-2 cells retain their capacity of differential gene expression of OPG and RANKL in favor of OPG after exposure to silicate. It is concluded that after exposure of the cells to silicate a factor(s) is released from SaOS-2 cells that causes a significant inhibition of osteoclastogenesis of RAW 264.7 cells. It is assumed that it is an increased secretion of the cytokine OPG that is primarily involved in the reduction of the osteoclastogenesis of the RAW 264.7 cells. It is proposed that silicate might have the potential to stimulate osteogenesis in vivo and perhaps to ameliorate osteoporotic disorders.

Open Orthop J. 2012;6:143-9. **Essential Nutrients for Bone Health and a Review of their Availability in the Average NorthAmerican Diet.**

Price CT[1], Langford JR, Liporace FA.

Abstract

Osteoporosis and low bone mineral density affect millions of Americans. The majority of adults in North America have insufficient intake of vitamin D and calcium along with inadequate exercise. Physicians are aware that vitamin D, calcium and exercise are essential for maintenance of bone health. Physicians are less likely to be aware that dietary insufficiencies of magnesium, silicon, Vitamin K, and boron are also widely prevalent, and each of these essential nutrients is an important contributor to bone health. In addition, specific nutritional factors may improve calcium metabolism and bone formation. It is the authors' opinion that nutritional supplements should attempt to provide ample, but not excessive, amounts of factors that are frequently insufficient in the typical American diet.In contrast to dietary insufficiencies, several nutrients that support bone health are readily available in the average American diet. These include zinc, manganese, and copper which may have adverse effects at higher levels of intake. Some multivitamins and bone support products provide additional quantities of nutrients that may be unnecessary or potentially harmful.The purpose of this paper is to identify specific nutritional components of bone health, the effects on bone, the level of availability in the average American diet, and the implications of supplementation for each nutritional component. A summary of recommended dietary supplementation is included.

Int J Endocrinol. 2013;2013:316783. **Silicon: a review of its potential role in the prevention and treatment of postmenopausal osteoporosis.**

Price CT[1], Koval KJ, Langford JR.

Abstract

Physicians are aware of the benefits of calcium and vitamin D supplementation. However, additional nutritional components may also be important for bone health. There is a growing body of the scientific literature which recognizes that silicon plays an essential role in bone formation and maintenance. Silicon improves bone matrix quality and facilitates bone mineralization. Increased intake of bioavailable silicon has been associated with increased bone mineral density. Silicon supplementation in animals and humans has been shown to increase bone mineral density and improve bone strength. Dietary sources of bioavailable silicon include whole grains, cereals, beer, and some vegetables such as green beans. Silicon in the form of silica, or silicon dioxide (SiO2), is a common food additive but has limited intestinal absorption. More attention to this important mineral by the academic community may lead to improved nutrition, dietary supplements, and better understanding of the role of silicon in the management of postmenopausal osteoporosis.

Biol Trace Elem Res. 2013 Apr;152(1):105-12. **Effects of silicon on osteoblast activity and bone mineralization of MC3T3-E1 cells.**

Kim EJ[1], Bu SY, Sung MK, Choi MK.

Abstract

Previous studies have reported that dietary silicon (Si) intake is positively associated with bone health including bone mineral density. Although the amount of Si intake is high among trace elements in humans, how dietary Si affects bone formation at the cellular level is not well addressed. The purpose of this study was to investigate the role of Si in osteoblast activity and bone mineralization. MC3T3-E1 was cultured as mature osteoblasts and treated with sodium metasilicate (0, 1, 5, 10, 25, 50, and 100 μM) as a source of Si. After 7 days of treatment, 5 and 10 μM of sodium metasilicate significantly increased intracellular alkaline phosphatase activity ($p < 0.05$) when compared to the control. Additionally, all doses of sodium metasilicate (1, 5, 10, 25, 50, and 100 μM) increased mineralized nodule formation at 14 days of differentiation as evidenced by increased Alizarin Red S staining. In the analysis of gene expression, 50 μM of sodium metasilicate upregulated type I collagen (COL-I) compared to the control group. However, the increase of COL-I gene expression as a result of treatment with 1, 10, 25, and 100 μM of sodium metasilicate did not reach statistical significance. mRNA expression of insulin-like growth factor-I and receptor activator of NF-κB ligand was not significantly changed at any dose of sodium metasilicate (0, 1, 5, 10, 25, 50, and 100 μM). In light of the results, we conclude that Si has a positive effect on bone metabolism by enhancing osteoblast mineralization activity.

J Nutr Health Aging. 2014 Nov;18(9):820-6. **A review of the effects of dietary silicon intake on bone homeostasis and regeneration.**

Rodella LF[1], Bonazza V, Labanca M, Lonati C, Rezzani R.

Abstract

Objective: Increasing evidences suggest that dietary Silicon (Si) intake, is positively correlated with bone homeostasis and regeneration, representing a potential and valid support for the prevention and improvement of bone diseases, like osteoporosis. This review, aims to provide the state of art of the studies performed until today, in order to investigate and clarify the beneficial properties and effects of silicates, on bone metabolism. Methods: We conducted a systematic literature search up to March 2013, using two medical databases (Pubmed and the Cochrane Library), to review the studies about Si consumption and bone metabolism. Results: We found 45 articles, but 38 were specifically focused on Si studies. Conclusion: Results showed a positive relationship between dietary Si intake and bone regeneration.

Stem Cells Int. 2016;2016:5653275. **Bone Anabolic Effects of Soluble Si: In Vitro Studies with Human Mesenchymal Stem Cells and CD14+ Osteoclast Precursors.**

Costa-Rodrigues J[1], Reis S[1], Castro A[1], Fernandes MH[1].

Abstract

Silicon (Si) is indispensable for many cellular processes including bone tissue metabolism. In this work, the effects of Si on humanosteogenesis and osteoclastogenesis were characterized. Human mesenchymal stem cells (hMSC) and CD14+ stem cells, as osteoblast and osteoclast precursors, were treated with a wide range of Si concentrations, covering the physiological plasma levels. Sipromoted a dose-dependent increase in hMSC proliferation, differentiation, and function, at levels similar to the normal basal plasma levels. Additionally, a decrease in the expression of the osteoclastogenic activators M-CSF and RANKL was observed. Also, Si elicited a decrease in osteoclastogenesis, which became significant at higher concentrations, as those observed after meals. Among the intracellular mechanisms studied, an upregulation of MEK and PKC signalling pathways was observed in both cell types. In conclusion, Si appears to have a direct positive effect on human osteogenesis, at basal plasma levels. On the other hand, it also seemed to be an inhibitor of osteoclastogenesis, but at higher concentrations, though yet in the physiological range. Further, an indirect effect of Si on osteoclastogenesis may also occur, through a downregulation of M-CSF and RANKL expression by osteoblasts. Thus, Si may be an important player in bone anabolic regenerative approaches.

Biol Trace Elem Res. 2016 May;171(1):138-44. **Effect of Silicon Supplementation on Bone Status in Ovariectomized Rats Under Calcium-RepleteCondition.**

Bu SY[1], Kim MH[2], Choi MK[3].

Abstract

Previous studies have suggested that silicon (Si) had positive effects on bone, but such benefits from Si may be dependent on calcium status. Also, several biochemical roles of Si in osteoblastic mineralization, the regulation of gene expression related to bone matrix synthesis, and the decrease in reactive oxygen species and pro-inflammatory mediators were reported, but these effects were mostly shown in cell culture studies. Hence, we tested the effect of Si supplementation on bone status and the gene expression related to bone metabolism and inflammatory mediators in young estrogen-deficient rats under calcium-replete condition (0.5 % diet). Results showed that 15-week supplementation of both high and very high doses of Si (0.025 and 0.075 % diet, respectively) could not restore the ovariectomy (OVX)-induced decrease of bone mineral density (BMD) of vertebrae, femur, and tibia. Also,

several bone biochemical markers (ALP, osteocalcin, CTx) and mRNA expression of COL-I, RANKL, IL-6, and TNF-α in femur metaphysis were not significantly changed by Si in OVX rats. However, a very high dose (0.075 %) of Si supplementation significantly increased OPG expression and decreased the ratio of RANKL/OPG in mRNA expression comparable to that of sham-control animals. Taken together, Si supplementation did not increase BMD under calcium-replete condition but the decrease in the ratio of RANKL/OPG expression to the normal level suggests the possibility of a bone health benefit of Si in estrogen deficiency-induced bone loss.

Acta Biomater. 2016 Jul 15;39:192-202. **Orthosilicic acid, Si(OH)4, stimulates osteoblast differentiation in vitro by upregulating miR-146a to antagonize NF-κB activation.**

Zhou X[1], Moussa FM[2], Mankoci S[3], Ustriyana P[3], Zhang N[4], Abdelmagid S[2], Molenda J[5], Murphy WL[5], Safadi FF[6], Sahai N[7].

Abstract

Accumulating evidence over the last 40years suggests that silicate from dietary as well as silicate-containing biomaterials is beneficial to bone formation. However, the exact biological role(s) of silicate on bone cells are still unclear and controversial. Here, we report that orthosilicic acid (Si(OH)4) stimulated human mesenchymal stem cells (hMSCs) osteoblastic differentiation in vitro. To elucidate the possible molecular mechanisms, differential microRNA microarray analysis was used to show that Si(OH)4 significantly up-regulated microRNA-146a (miR-146a) expression during hMSC osteogenic differentiation. Si(OH)4 induced miR-146a expression profiling was further validated by quantitative RT-PCR (qRT-PCR), which indicated miR-146a was up-regulated during the late stages of hMSC osteogenic differentiation. Inhibition of miR-146a function by anti-miR-146a suppressed osteogenic differentiation of MC3T3 pre-osteoblasts, whereas Si(OH)4 treatment promoted osteoblast-specific genes transcription, alkaline phosphatase (ALP) production, and mineralization. Furthermore, luciferase reporter assay, Western blotting, enzyme-linked immunosorbent assay (ELISA), and immunofluorescence showed that Si(OH)4 decreased TNFα-induced activation of NF-κB, a signal transduction pathway that inhibits osteoblastic bone formation, through the known miR-146a negative feedback loop. Our studies established a mechanism for Si(OH)4 to promote osteogenesis by antagonizing NF-κB activation via miR-146a, which might be interesting to guide the design of osteo-inductive biomaterials for treatments of bone defects in humans.

STATEMENT OF SIGNIFICANCE:

Accumulating evidence over 40years suggests that silicate is beneficial to bone formation. However, the biological role(s) of silicate on bone cells are still unclear and controversial. Here, we report that Si(OH)4, the simplest form of silicate, can stimulate human mesenchymal stem cells osteoblastic differentiation. We identified that miR-146a is the expression signature in bone cells treated with Si(OH)4. Further analysis of miR-146a in bone cells reveals that Si(OH)4 upregulates miR-146a to antagonize the activation of NF-κB. Si(OH)4 was also shown to deactivate the same NF-κB pathway to suppress osteoclast formation. Our findings are important to the development of third-generation cell-and gene affecting biomaterials and suggest silicate and miR-146a can be used as pharmaceuticals for bone fracture prevention and therapy.

Biol Trace Elem Res. 2016 Oct;173(2):306-15. **Biological Silicon Stimulates Collagen Type 1 and Osteocalcin Synthesis in Human Osteoblast-Like Cells Through the BMP-2/Smad/RUNX2 Signaling Pathway.**

Dong M[1], Jiao G[1], Liu H[1], Wu W[1], Li S[1], Wang Q[1], Xu D[1], Li X[1], Liu H[1], Chen Y[2].

Abstract

Silicon is essential for bone formation. A low-silicon diet leads to bone defects, and numerous animal models have demonstrated that silicon supplementation increases bone mineral density (BMD) and reduces bone fragility. However, the exact mechanism of this action has not been characterized. In this study, we aimed to determine the role of biological silicon in the induction of osteoblast differentiation and the possible underlying mechanism. We examined whether orthosilicic acid promotes collagen type 1 (COL-1) and osteocalcin synthesis through the bone morphogenetic protein-2 (BMP-2)/Smad1/5/runt-related transcription factor 2 (RUNX2) signaling pathway by investigating its effect in vitro at several concentrations on COL-1 and osteocalcin synthesis in human osteosarcoma cell lines (MG-63 and U2-OS). The expression of relevant proteins was detected by Western blotting following exposure to noggin, an inhibitor of BMP-2. In MG-63 cells, immunofluorescence methods were applied to detect changes in the expression of BMP-2, phosphorylated Smad1/5 (P-Smad1/5), and RUNX2. Furthermore, rat bone mesenchymal stem cells (BMSCs) were used to determine the effect of orthosilicic acidon osteogenic differentiation. Exposure to 10 μM orthosilicic acid markedly increased the expression of BMP-2, P-Smad1/5, RUNX2, COL-1, and osteocalcin in osteosarcoma cell lines. Enhanced ALP activity and the formation of mineralized nodules were also observed under these conditions. Furthermore, preconditioning with noggin inhibited the silicon-induced upregulation of P-Smad1/5, RUNX2, and COL-1 expression. In conclusion, the BMP-2/Smad1/5/RUNX2 signaling pathway participates in the silicon-mediated induction of COL-1 and osteocalcin synthesis, and orthosilicic acid promotes the osteogenic differentiation of rat BMSCs.

Journal of Arthroscopy and Joint Surgery : September–December 2017, Volume 4, Issue 3, Pages 103-105.**The Promise of Silicon: bone regeneration and increased bone density**

M. Aroraa,*, E. Arorab

Abstract

Historically, silicon has been accorded low importance as a trace element. Its role has generally been regulated to that of minor influence on bone and connective tissue development. However, in vitro and in vivo studies have shown that these assumptions are incorrect. Silicon plays a key role in bone biology, improving bone regeneration and increasing bone mineral density. The aim of this review is to provide an understanding of the role of silicon in bone biology and its clinical application.

Silicon has a key role to play in bone biology. Although poorly understood, its mechanism of action is likely related to the synthesis and stabilization of collagen in bone matrix. In vivo and in vitro studies have demonstrated its importance in improving bone regeneration and increasing bone mineral density. Clearly, this forgotten element holds much promise for the future of orthopaedics.

Pharmaceutics. 2019 Mar 12;11(3). **Effects of Silicon Compounds on Biominera lization, Osteogenesis, and Hard Tissue Formation.**

Götz W[1], Tobiasch E[2], Witzleben S[3], Schulze M[4].

Abstract

Bioinspired stem cell-based hard tissue engineering includes numerous aspects: The synthesis and fabrication of appropriate scaffold materials, their analytical characterization, and guided osteogenesis using the sustained release of osteoinducing and/or osteoconducting drugs for mesenchymal stem cell differentiation, growth, and proliferation. Here, the effect of silicon- and silicate-containing materials on osteogenesis at the molecular level has been a particular focus within the last decade. This review summarizes recently published scientific results, including material developments and analysis, with a special focus on silicon hybrid bone composites. First, the sources, bioavailability, and functions of silicon on various tissues are discussed. The second focus is on the effects of calcium-silicate biomineralization and corresponding analytical methods in investigating osteogenesis and bone formation. Finally, recent developments in the manufacturing of Si-containing scaffolds are discussed, including in vitro and in vivo studies, as well as recently filed patents that focus on the influence of silicon on hard tissue formation.

J Biomater Appl. 2019 Jul;34(1):94-103. **The role of orthosilicic acid-induced autophagy on promoting differentiation and mineralization of osteoblastic cells**

Hai Chi, Meng Kong, Guangjun Jiao, ...

Abstract

Silicon, an element, has been reported to possess various osteogenic biological functions. However, the precise cellular action mechanism of silicon in osteogenesis is elusive. Here, we examine whether autophagy is associated with the osteogenic differentiation and mineralization of preosteoblasts stimulated by orthosilicic acid (unique soluble form of silicon). The autophagy induced by silicon was detected via autophagy-related protein expression. The correlation between orthosilicic acid-induced autophagy and the osteogenic process was verified by the autophagy inhibitor 3-methyladenine. We also investigated its applicability in vivo through bone mineral density, collagen staining, hydroxyproline, and immunohistochemistry on osteoporosis rats. Concentrations above 10 µM increased alkaline phosphatase activity, whereas those up to 50 µM decreased alkaline phosphatase activity. Orthosilicic acid at concentrations up to 20 µM increased the osteogenetic indicators, the expression of the autophagy-related factors SQSTM1/p62, and the conversion of microtubule-associated protein 1 light chain 3-II (LC3-II) from microtubule-associated protein 1 light chain 3-I (LC3-I) according to Western Blot analyses. The application of 3-methyladenine decreased the expression of Runt-related transcription factor 2 (RUNX-2), bone morphogenetic protein-2, and collagen I in the Western Blot analysis. Low dose of orthosilicic acid supplemented rats (OVX-1, 2) showed improvements in such parameters as the expression rate of collagenous fiber in bone, bone mineral density, femoral hydroxyproline content, and bone morphogenetic protein-2; while high dose groups (OVX-3, 4) presented no better outcome. The present study showed that orthosilicic acid stimulated the osteogenic differentiation and mineralization of cultured preosteoblasts by inducing autophagy. In addition, it also can enhance bone mineral density of osteoporosis rats.

Biol Trace Elem Res. 2019 Aug;190(2):327-335. **Orthosilicic Acid Accelerates Bone Formation in Human Osteoblast-Like Cells Through the PI3K-Akt-mTOR Pathway**.

Zhou H[1,2], Jiao G[1], Dong M[3], Chi H[1], Wang H[1], Wu W[1], Liu H[1], Ren S[1], Kong M[1], Li C[1], Zhang L[1], Chen Y[4,5].

Abstract

Silicon is one of the essential trace elements in the human body; the deficiency of which may lead to bone diseases. Numerous animal experiments have shown that an appropriate increase in the intake of silicon is beneficial to enhancing bone density and toughness to prevent osteoporosis. However, the molecular mechanisms of the silicon-mediated osteogenesis process have not been sufficiently clarified. In this study, we determined the possible osteogenesis-related mechanisms of orthosilicic acid at a molecular level. We detected the relevant pathway and osteogenic indicators by immunofluorescence (IF), Western blot, alkaline phosphatase (ALP) staining (using 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium [BCIP/NBT]), ALP enzyme labeling method, osteocalcin (OCN), and N-terminal propeptide of type 1 procollagen (P1NP) enzyme-linked immunosorbent assay (ELISA). We found that orthosilicic acid is capable of enhancing the expression of phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), phospho-protein kinase B (P-Akt), phospho-mammalian target of rapamycin (P-mTOR), and related osteogenic markers (runt-related transcription factor 2 [RUNX2], type I collagen [COL1], ALP, OCN, and P1NP). However, with the addition of PI3K-Akt-mTOR pathway-specific inhibitor LY294002, the expression of PI3K, P-Akt, P-mTOR, RUNX2, COL1, ALP, OCN, and P1NP decreased. The results indicated that the PI3K-Akt-mTORpathway played a positive regulatory role in the process of orthosilicic acid-mediated osteogenesis in vitro.

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