SİLİKON İKAMELİ HİDROKSİAPATİTTE BİYOAKTİVİTE VE KEMİK OLUŞUMU

Ulvan Ozad[1], Alan Parish[2], Karin A. Hing[2]

[1] Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London E1 2AD, UK
[2] Department of Materials, IRC in Biomaterials, Queen Mary University of London, London E1 4NS, UK

ÖZET

Silikon ikameli hidroksiapatit kemik graftlarındaki biyoaktivite ve başarılı kemik ulaşımını ölçmek amacıyla taramalı elektron mikroskobu ve electron dağınımlı x-ray ışınları görünge gözlemi ile incelenmiştir. Taramalı elektron mikroskobu sonuçlarında kemik oluşum alanları saptanmış, düzenli lamellar kolajen gözlemlenmiştir. Kemik graftı ve üretilmiş kemik arasında 20.8% avaraj karbon içerik artışı saptanmış ve bu interfaz boyunca aşamalı olan artış ile doğrulanmıştır. Bariz kemik oluşumu ve olgunlaşması gözlemlenmiştir. Karbon miktarı kemik graftından yeni oluşan kemik dokusuna doğru aşamalı olarak artış göstererek yeni kemik dokusu oluşumunu ve silikon ikameli hidroksiapatit çözülümünü doğrulamıştır.

Anahtar Kelimeler: Silikon, hidroksiapatit, kemik, karbon, nano

BIOACTIVITY AND BONE FORMATION IN SILICON-SUBSTITUTED HYDROXYAPATITE

ABSTRACT

Bioactivity and successful bone formation in silicon-substituted hydroxyapatite bone grafts were investigated by using scanning electron microscopy and electron dispersive x-ray spectroscopy. Areas of bone formation have been detected in scanning electron microscopy; and, arranged lamellar collagen has been observed. 20.8% average carbon content rise has been detected between bone graft and the produced bone; and, this has been confirmed to be a gradual increase throughout the interphase. Obvious bone formation and maturation were observed in the samples. Carbon content gradually increased from bone graft to the bone formed, confirming formation of new bone and dissociation of silicon-substituted bone graft.

Keywords: Silicon, hydroxyapatite, bone, carbon, nano

1. INTRODUCTION

Bone is the major element of skeleton. Cortical and trabecular bones demonstrate variations in their structure. The unit structures of cortical bone, osteons (haversian systems), receive the blood and nervous supply by endosteum[1, 2]. Interstitial lamella, in bone, is irregular and is formed by remodeling of osteons between haversian systems[3]; and, the attachment to outer structures is held by periosteum[2]. The inorganic component of bone provides resistance and stiffness to the forces applied. Bone mineral is the main component of bone and possesses similar crystallographic structure with hydroxyapatite; however, the internal crystallinity is disordered causing existence of trace elements such as sodium, bicarbonate and magnesium[4, 5, 6, 7]. The calcium to phosphorus ratio of hydroxyapatite is 1.67 whereas this ratio could change from 1.37 to 1.87 in bone[8, 9, 6]. Bone mineral and collagen together create the rigid and hard nature of bone[2]. Collagen type 1 is the major collagen type present in bone and carbon is the main element making up the collagen backbone. Conditions influencing osteogenesis such as loading could cause variations in the collagen fibre orientation[10] as well as the osteogenesis itself; and, these lead to woven and lamellar bone formation. Woven bone is the primary, immature bone formed when fast bone formation leads to irregular collagen deposition and is observed in fetus, fractures and pathological conditions[11]. Lamellar bone is the secondary, mature bone formed with collagen organized into lamellar arrangement[12]. Mineralized structure of the bone do not allow oxygen and nutrient diffusion; therefore, vascularization is necessary for formation and repair[6]. Remodelling rate is different in trabecular (25% per year) and cortical (3% per year) bones[13].

Theories have been present on mechanisms initiating and causing bone remodeling. Julius Wolff states that modification of bone tissue takes place according to the loading; and, increased stress on bone leads to stronger bone formation[14]. Harold Frost points out the mechanostat model which takes the force applied on bone by muscles as the principle for adaptation to elastic deformation on bone taking place[15]. Healing of bone takes place by endochondral ossification where initially cartilage tissue and callus is formed, then is converted to woven bone[6]. Structure of bone and processes of formation and healing is important for selection and application of bone grafts.

Bone grafting is used in conditions where surgical devices are implanted such as joint replacement, fast healing of fracture, bone regeneration after bone loss or certain pathological conditions requiring arthrodesis is required.

In bone graft selection, the properties of bone grafts are very important as they will directly influence the prognosis. Bone is a living tissue and it could completely regenerate in feasible conditions; therefore, these conditions should be supplied by the implanted bone graft. The ideal bone graft should possess osteointegration, osteoinduction, osteogenesis and osteoconduction. Osteointegration is the direct implant surface and bone bonding[16], osteoinduction is promotion of osteoblast differentiation from pluripotent stem cells[17], osteogenesis is the bone growth performed by osteoblasts arriving from the implant; and, osteoconduction is supplying a template scaffold for attachment in order to enable growth[18]. In non-critical defect sizes, grafts with only osteoconductive properties could be used but in large defects, osteoinduction or osteogenesis are also required[18, 19]. Autologous bone grafts are the self grafts, typically obtained from the iliac crest of patient, but these grafts are limited in availability and lead to donor site morbidity. Allogenic bone grafts are non-self human bone grafts and the availability is not problem but there is risk of disease transmission[20] and immunological response[21] leading to fractures and graft failure. Synthetic bone grafts are artificially produced biocompatible grafts. The main synthetic bone grafts are glass ionomers and bioactive glasses, calcium sulphates and calcium phosphates.

Calcium phosphates possess osteointegrity and osteoconductivity. Beta tricalcium phosphate is a type of calcium phosphate bone graft but fast resorption of the graft, before bone formation and defect repair, makes this graft undesirable[22]. Hydroxyapatite is also a calcium phosphate bone graft, similar to bone mineral, available in powder, granule or solid block forms[23].
Hydroxyapatite is bioactive, forming interfacial bonding between bone and graft[24], possess both osteoconductive and osteoinductive properties[25], and provide a feasible environment for bone growth. Dissolution of calcium and phosphate leads to increased ionic concentration in bone-graft interphase and this enables apposition of bone on the graft [26].

Pore interconnectivity which involves both the size of pores and extent of porosity is influential in the success of bone grafts[6] because it supports revascularization, leading to vessel formation for oxygen and nutrient delivery. Porosity increases the surface area and allows cellular infiltration. The lower threshold for porosity is 60%[27, 28] and increasing the macroporosity improves the bone ingrowth[29] but porosity above 80% provides decreased load bearing

capacity and faster resorption therefore is not feasible. Microporosity of the graft should be minimum between 45 and 100 micrometres[30]. Increasing the microporosity increases the bioactivity and leads to faster bone growth; however, this does not change the quality of bone formed because there is no difference in osteoblasts[31].
The biological response of body is affected by roughness, chemistry and physiology of implant surface. Substitution of elements in hydroxyapatite modifies the lattice and the chemical structure which in turn influences the solubility and tissue response[32]. In 1970, Carlisle detected deformities in bones and decreased weight gain in chicks having silicon-deficient diet[33]. In 1972, Schwars and Milne proved that the body needs small amounts of silicon by observing the deformations in skull and impaired growth in mice when dietary silicon is deficient[34]. Substitution of phosphate with silicon or carbonate improves the solubility and bioactivity of the graft[35]. Such grafts are formed by substitute precipitation followed by filtration, drying, milling and sintering at temperatures between 975 and 1300°C[36]. Orthosilicic acid presence improves collagen type 1 synthesis and cell differentiation[37, 38, 28]. Silicon substitution in hydroxyapatite bone graft initiates two mechanisms: active and passive. In active mechanism, release of silicon takes place leading to improved solubility and detection of silicon by cells. In passive mechanism, increased boundaries of grains and negative charge of surface improves the protein attachment[39, 40]. The quantity of silicon affects the charge of surface, pH and wettability. 0.8 weight percent silicon is known to improve the protein adsorption and cell adhesion by improving the surface[41, 42]. Silicon substituted hydroxyapatite grafts perform better dissolution and bone apposition [43, 28, 40] by liberation of silicon and alteration of the topography leading to adhesion of peptides and osteoblasts[44]. Stable graft resorption offers enough time for angiogenesis, formation of bone and remodeling of bone according to anatomical requirements and applied loading[45].

Post operative complaints and complications are nerve damage, risk of infection, blood loss, stiffness and pain; all non-specific to bone grafting[46]. The management during healing is easy procedures such as elevation, painkillers and cold-hot pack applications. The healing duration depends on type and size of implants, usually between 2 weeks and 3 months; however, actual repair of the graft site lasts for over three months[47].

2. MATERIALS AND METHODS

Two silicon-substituted hydroxyapatite bone grafts with 30% strut porosity and 80% total porosity were embedded in para-spinal muscles of sheep at first lumbar vertebra level. Sheep were sacrificed at twelve weeks after intramuscular implantation and the graft areas were provided for this study in resin-embedded form. These samples were prepared for scanning electron microscopy (SEM) and electron dispersive x-ray spectroscopy (EDS) examination by polishing with 1200, 2400 and 4000 grade silicon carbide sand papers in polishing machine, mounting on sample holder with carbon paint and gold coating.

INCA software was used, with 10cm working distance and 20kV charging, in SEM. In EDS, bone graft-bone interphase was found, bone presence was confirmed by detecting collagen in high magnifications, and the interphase was recorded at 6000 times magnification. 3x5 dot-matrix with equal horizontal and vertical spacing was drawn on the interphase and the quantitative data on elemental content was obtained from each data point by investigating the element peaks in spectroscopy, by using quant function of INCA. This was repeated on five different interphases and horizontal means were taken to find average elemental carbon content of the bone, bone graft and interlayer in each sample. The accuracy level of these measurements was 0.1%. Bone maturity was investigated by taking SEM pictures of collagen layers in bone at high magnifications.

3. RESULTS

Amount of carbon (atomic percent) in bone graft and the bone produced were determined by EDS. The table below (Table 1) demonstrates atomic percent values of carbon in Sample 1 and Sample 2 (both silicon-substituted hydroxyapatite 80/30) with standard deviations. Data point 1 demonstrates the bone produced, data point 5 demonstrates the bone graft and the points between these represent the interphase where gradual bone production is present.

Table 1: Carbon content of samples

Data Point	Atomic %	
	Sample 1	Sample 2
1 (Bone Produced)	41.54±3.52	47.33±2.75
2	40.76±2.86	50.71±5.31
3	30.23±5.88	41.14±4.94
4	22.95±2.21	27.66±3.81
5 (Bone Graft)	21.14±2.14	26.23±4.44

In both samples, the amount of carbon increased going from bone graft to bone and as observed from the table, this increase is gradual. In sample 1, atomic carbon percent increased from 21.14±2.14% to 41.54±3.52% and in sample 2, atomic carbon percent increased from 26.23±4.44% to 47.33±2.75%.

The bone graft success, in terms of the bone produced, could be examined by the difference of atomic carbon contents between data points 1 (bone) and 5 (bone graft). The figure 1 bar chart below demonstrates the carbon content difference between the bone graft and the bone produced. Atomic percentage differences of carbon means and error bars representing standard deviations are provided in the Figure 1.

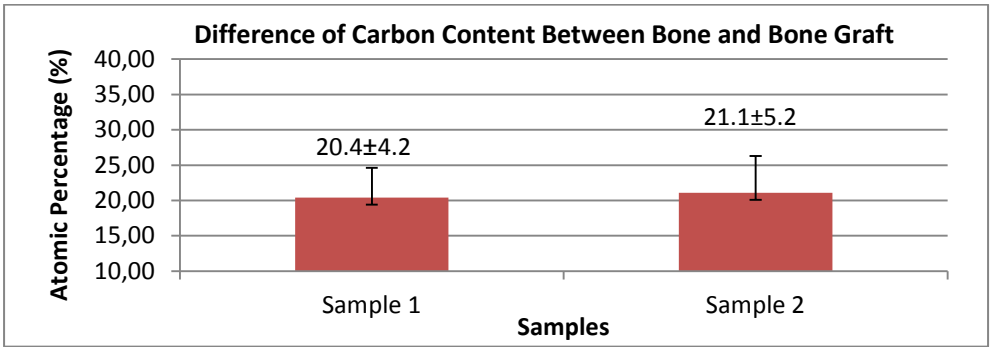

Figure 1: Difference of atomic carbon percentages between bone and bone graft

Average carbon difference between the graft and bone produced was found to be 20.8%. One-way ANOVA Tukey HSD test was performed and no significant difference was found between the carbon content differences of samples.

SEM images with high magnification were obtained to evaluate the presence and maturity by examining the collagen alignment and organization. Presence of collagen in layers was considered as mature (lamellar) bone whereas unorganized collagen was considered as woven bone.

The SEM image below (Figure 2) is the photo taken from sample 1 at 50000 times magnification. The area expressed by arrow shows the overlying collagen layers which prove that remodeling into mature bone took place; therefore, sample 1 illustrated the presence of mature bone.

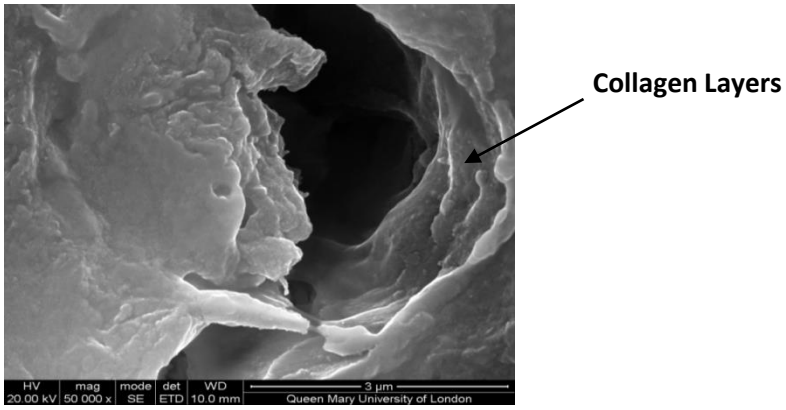

Figure 2: High magnification SEM image of sample 1

The SEM image below (Figure 3) was taken from the sample 2 at 50000 times magnification. The area expressed by arrow is the collagen layers which were organized regularly and in parallel direction to create a strong structure; therefore, sample 4 had mature (lamellar) bone.

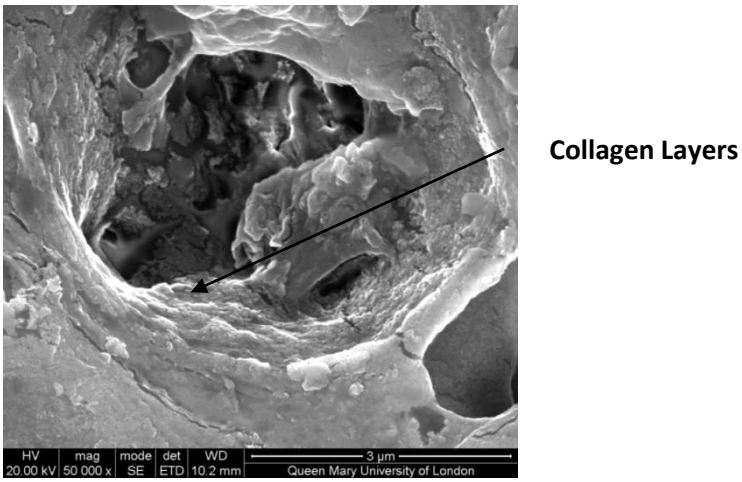

Figure 3: High magnification SEM image of sample 2

4. DISCUSSION

Samples had porosity above 60% which is accepted as the baseline porosity for supporting bone growth. In the elemental content analysis, both samples demonstrated a gradual increasing pattern from bone graft to the bone. There is no carbon in hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) bone graft, atomic carbon percentage present in the graft is the carbon present in resin, where the sample was embedded in; and, this would be the baseline of carbon amount present. Carbon is present in collagen backbone in high amounts[48]. Collagen is present in high amounts in bone and when going from bone graft to bone, collagen amount and remodeling (organization) increases together with increased amount of bone produced; therefore, carbon amount also increases. Both of the samples had similar trends and values and no significant difference in carbon content differences. This demonstrates that samples with same strut and total porosities, despite having differences in sintering or other production parameters, possess similar elemental content results.

In bone maturity analysis, remodeling into mature bone in 12 weeks was observed in both samples. Presence of layered collagen not only proved presence of remodelled bone, but also eliminated the possibility of the new growing tissue being and staying as callus.

Previous studies finding better bone growth in silicon-substituted hydroxyapatite bone grafts are present[49, 50, 51] and measuring the elemental content of bone mineral[52, 53, 54, 24,] there was lack of literature on determining the bone elemental content, growth and bioactive nature by EDS atomic percent elemental contents. Also, despite studies were present on other bone maturity determination methods such as infrared spectroscopy[55] and EDS[56] and imaging of collagen and bone were widely performed, no studies were present to determine the mature bone in bone-bone graft interphase by high magnification SEM.

The major limitations present in this study were the dearth of research on the methods used, biomechanics and characteristics of sheep bone demonstrating variations compared to human bone and image shifting taking place during the recording of elemental contents in EDS.

5. CONCLUSION

Bone growth was successfully detected by increase of atomic carbon percent content in the bone tissue formed compared to the bone graft measured by EDS, and presence of remodelled, mature bone was confirmed by high magnification SEM imaging. Carbon content was gradually increasing from the graft to bone, confirming increase of collagen and formation of new bone.

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