Medical progress

Osteostimulation of bioglass

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Bioglass, with the code name 45S5, contains 45% silica, 24.5% CaO, 24.5% Na_2O and 6% P_2O_5 in weight percent. All of these oxides are mixed together and melted at around 1350°C to form a homogeneous silica network glass.[1] Bioglass possesses fast biological response and fast bioactivity, when implanted in living tissue. Since Hench et al[1] reported over 35 years ago that the bioglass composition could chemically bind to bone, the research and development of bioactive glasses has been a very active field, involving many research groups worldwide and tens of thousands of studies on bioactive glasses have been published. Still, so far, no other bioactive glass composition has been reported that demonstrates a faster biological response than bioglass.

Bioglass has been used as a synthetic bone graft material, with two products developed and used clinically for over 10 years in the US, Europe and China: NovaBone, a bone graft product used in the orthopedic field, and PerioGlas, a bone graft product used in dental and maxilofacial surgery. In 2005, the bioglass products were cleared by the US Food and Drug Administration (FDA) for osteostimulation: "The stimulation of osteoblast proliferation and differentiation as evidenced during *in vitro* osteoblast cell culture studies by increased DNA content and elevated osteocalcin and alkaline phosphatase levels". It has been over 35 years from the first report of chemical bonding to bone to the now recognized bone stimulation function of bioglass.

For a bone graft material, osteoinduction, osteostimulation and osteoconduction are 3 levels of biological response with osteoinduction being the highest level. The osteoinductive graft materials such as bone morphogenetic protein (BMP) and autogenous bone are able to induce stem cell to bone cell transition and are supposed to promote new bone growth in the bony defect area at a fast rate. Osteostimulation is an intermediate level. The osteostimulative graft material can enhance the production of growth factors and promote the proliferation and differentiation of bone cells, which stimulate new bone formation and growth, and new bone can be seen simultaneously at the edge and center of the defect area. Osteoconductive graft materials can serve as a scaffold and the native bone grows along their surface from the edge of the defect to the center. A number of bioceramics such as calcium phosphate and calcium sulfate belong to this group. The accumulated evidence demonstrates that bioglass is both osteoconductive and osteostimulative.

This article reviews more than 35 years of research from *in vitro*, cell culture to animal studies of bioglass and the present evidence indicating its osteostimulative property.

IN VITRO SURFACE REACTION STUDY

In their first published article in 1971, Hench et al[1] reported that the bioglass composition could chemically bond to bone and its fast surface reaction was attributed to the bonding ability. The surface reaction takes place immediately upon bioglass contacting a physiological environment such as simulated body fluid (SBF)[2] or body fluid. The surface reaction includes the ion exchange between Na^+ from bioglass and H^+ from the fluid, and the formation of a porous silica rich layer on the surface, then, followed by the formation of hydroxyl-carbonate-apatite (HCA), a similar mineral phase of bone, on the bioglass surface.[3-11]

For over 20 years from 1971, the research focused mainly on the correlation of the surface reaction of bioglass with bone bonding, and it was found that the formation of the HCA layer was essential for bone bonding. If no HCA formed on the bioactive glass compositions there would be no bone bonding for those compositions either.[3,8] Bioglass showed fast HCA formation and also demonstrated fast and strong bone bonding.[8] In one study,[12] bioglass disks were reacted in Tris buffer solution, one kind of physiological solution, with type I collagen fibers up to 7 days. Figure 1 is the photograph of scanning electron microscopy (SEM) of the bioglass surface after day 1 and day 7 in the solution. Clearly, the collagen fibers attached to the bioglass surface and the HCA formed on the surface embedding the collagen fibers. This demonstrated the bonding process of the bioglass surface to bone, and also demonstrated a similar process of bone formation. The discovery was very encouraging because the process occured without the existence of bone cells. The result for the bone bonding

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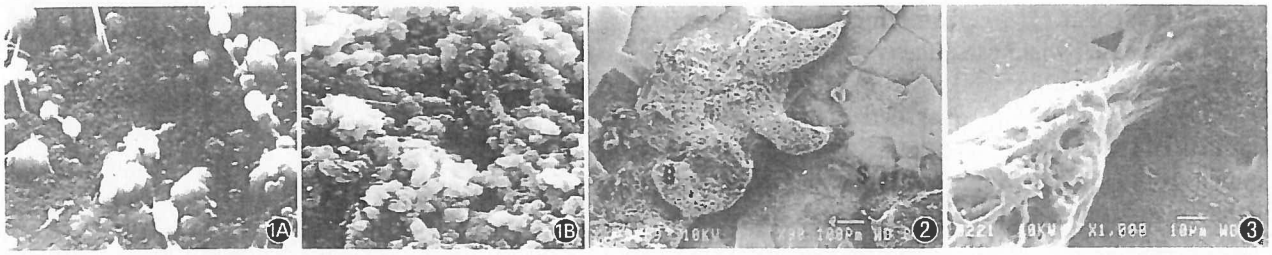

Figure 1. SEM of bioglass surface after reacting in Tris/collagen fibers solution (From the file of and permitted by NovaBone Products, LLC, USA. Original magnification). **A**: Day 1. **B**: Day 7.
Figure 2. Mineralized bone attached on the bioglass surface after 22 days osteoblast culture (Courtesy of Dr. JM Sautier, Laboratoire de Biologie, Univ Paris, France)
Figure 3. Bone nodular formed on bioglass surface after 22 days osteoblast culture (Courtesy of Dr. JM Sautier, Laboratoire de Biologie, Univ Paris, France)

and bone formation could be better if bioglass was placed under living conditions, and it was assumed that bioglass could have great osteoconductive ability.

In 2002, a study examined[13] the microstructure of bone-like apatite formed on the bioglass surface and also the bone-like apatite that was found in the physiological solution, SBF, when reacted with bioglass particles. This study again demonstrated the potential promotion of the mineralization process of bone formation by bioglass.

CELL CULTURE STUDY

Cell proliferation

Many articles have been published on bioglass cell culture.[14-17] Osteoblast proliferation and high activity of DNA synthesis were always reported in those articles. In 1993, Vrouwenvelder et al[16] reported that when bioglass was cultured with osteoblasts from 20-day-old fetal rats, it showed a better osteoblast character and higher mean proliferation rate than other materials such as hydroxylapatite (HA), titanium alloy and stainless steel. The alkaline phosphatase (ALP) activity was 3 times higher and DNA content was 35% more for bioglass cultures in 8 days compared to HA. Price et al[17] reported a higher proliferation rate and higher concentration of osteocalcin (OC) when osteoblasts were cultured with bioglass. All of the results, the higher ALP and OC production and higher DNA content, showed higher bone formation activity of osteoblasts when cultured with bioglass.

Actual bone formation has been observed by SEM on a bioglass surface when cultured with osteoblasts. After 22 days of culture, SEM examination revealed the mineralized bone attached to the bioglass surface (Figure 2) and also, it can be clearly seen that bone with nodular structure bonds well with bioglass (Figure 3).

Activation of gene expression

In 2000, Xynos et al[18] reported that the ionic products from bioglass degradation or dissolution not only increased the proliferation of human osteoblasts but also induced insulin-like growth factor II mRNA expression and protein synthesis. In his series of studies, Xynos et al[19] also reported the effect of the ionic products of bioglass 45S5 dissolution on the gene-expression profile of human osteoblasts by cDNA microarray analysis of 1176 genes. A total of 190 out of the 1176 genes surveyed in this study were shown to be expressed in human osteoblasts at levels above background. The expression of the genes from his works is partially listed in Table. The activation of osteoblast gene expression by bioglass from this study provided direct evidence to support the osteostimulation of bioglass at the molecular level. The activation of genes such as cell surface receptors, signal transduction molecules, growth factors, cell cycle regulators, matrix component, DNA synthesis etc, would accelerate the proliferation or growth of bone cells, hence, stimulate new bone formation and growth.

Table. Genes expression in human osteoblasts treated with the ionic products of bioglass dissolution 20

Genebank accession No.	Protein/Gene	Ratio	Function
M59040	CD44 antigen hematopoietic form precursor	7	Cell surface receptor
AF040105	RCL growth-related c-myc-responsive gene	5	Growth related gene
X59798	G1/S-specific cyclin D1	4	Cell cycle regulator
J03075	Protein kinase C substrate 80 kDa protein heavy chain	3.5	Signal transduction
U09579	Cyclin-dependent kinase inhibitor 1	3.4	Cell cycle regulator
M29645	Insulin-like growth factor II (IGF2)	3.2	Growth factor
X69391	60S ribosomal protein L6	3	Transcription
L42379	Bone-derived growth factor 1(BPGF1)	3	Growth factor
X79389	Glutathione S-transferase T1	3	Enzyme
J03210	Matrix metalloproteinase 2 (MMP2)	2.7	Matrix component
M14219	Decorin; bone proteoglycan II precursor	2.2	Matrix component
M11233	Cathepsin D precursor	2	Enzyme
X60188	Extracellular signal-regulated kinase 1	2	Signal transduction
AF060515	Cyclin K	2	Cell cycle regulator
L07541	Replication factor C 38-kDa subunit	2	DNA synthesis
J000123	Proenkephalin A precursor	2	Cell surface receptor

Change of cell growth cycle

As has been discussed above, bioglass can enhance the proliferation and differentiation of osteoblasts and produce a high content ALP and OC. Xynos et al[20] also reported in 2000 that the cell populations in both S phase

(DNA synthesis) and G2/M phase (mitosis) of the cell cycle were increased when osteoblasts were cultured with bioglass. As a continuous work, Sun et al[21] reported the same result and in his quantitative analysis the populations of the osteoblasts in both S phase and G2/M phase that were cultured with a bioglass solution reached a maximum in 2 days. In their study, human osteoblasts were isolated from trabecular bone of femoral heads from total hip arthroplasty, and the second passage of the osteoblasts was used. Compared with the osteoblasts cultured with just culture medium, in which, the S phase and G2/M phase reached a maximum in 4 days (Figure 4), bioglass solution achieved maximum cell division 2 days early as a result of a shorter cell growth cycle, and faster cell turnover. This result is coincident with the activation of cell cycle regulators by bioglass as discussed above. The short cell growth cycle and fast cell turnover again provide direct evidence for the osteostimulation of bioglass.

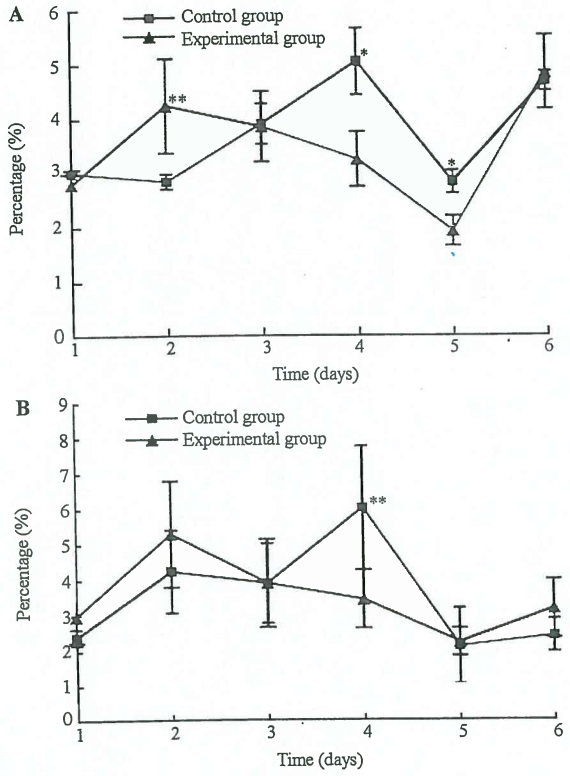

Figure 4. The distribution of osteoblasts in S phase (**A**) and G2/M phase (**B**)

ANIMAL STUDY

The osteostimulation of bioglass, observed in cell culture studies *in vitro*, can be directly reflected by fast and early bone formation in animal models. Although there have been several animal models, a typical model used for bioglass studies is the distal femur defect model in rabbits (Figure 5). In 1997, Oonishi et al[22] reported that, beginning at one week, bioglass generated new bone faster than HA; and in 6 weeks, new bone formed in the entire defect area with 100% new bone penetration into

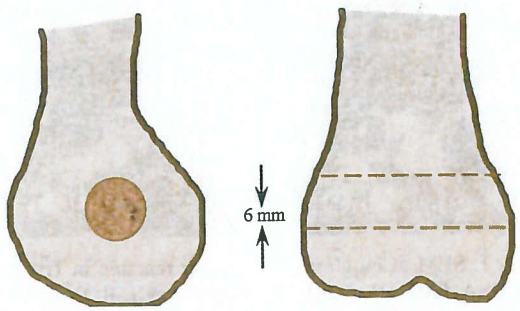

Figure 5. Transfemoral defect, 6 mm in diameter, in rabbit distal condoyle.

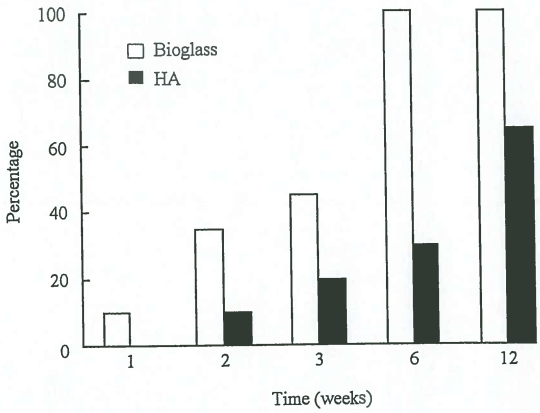

Figure 6. Percentage of new bone penetration into defect: bioglass vs HA.

the defect by bioglass (Figure 6), while there was only 30% new bone penetration into the defect by HA.

Chou et al[23] reported their study on the rabbit model using bioglass that new bone formation activity was found within 2 weeks of bioglass implantation, at this early stage of implantation bioglass stimulated osteogenic cells at the interface to generate a significantly higher level of osteocalcin expression, resulting in a remarkably increased bone formation.

In 2000, Wheeler et al[24] reported her rabbit study using bioglass and the results can be seen in the histological pictures in Figure 7. In Figure 7 B, the red color zone represents bone while G represents bioglass particles. Bioglass particles are distributed in the defect by implantation (Figure 7 A), and in 4 weeks (Figure 7 B), new bone (B) formed around the graft particles (G). In 12 weeks (Figure 7 C), significant new bone formed around the graft particles and in the whole defect area.

As a recent and future direction, the research with bioglass has already turned from direct osteoblast and bone tissue growth to its resorption,[25] antimicrobial,[26] antibacterial,[27] and anti-inflammatory,[28] potential and its application for tissue engineering.[29] Reports of more of this work can be expected in publications in the coming years.

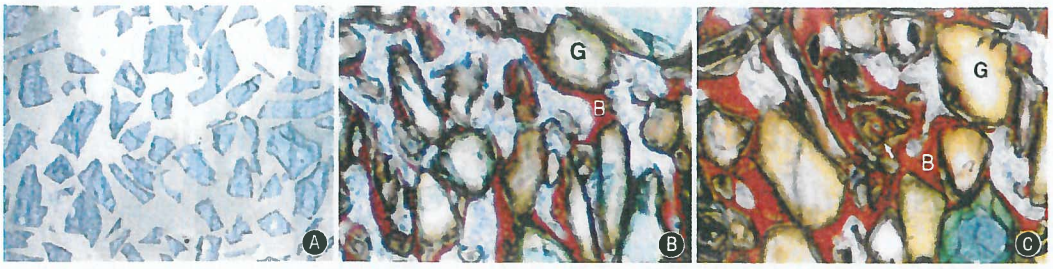

Figure 7. New bone formation stimulated by bioglass in rabbit model (Courtesy of Dr. D.L. Wheeler, Department of Orthopaedics, University of Florida, Gainesville, USA).

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