

Chapter 1

Bioactive Materials for Tissue Engineering Scaffolds

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INTRODUCTION

Millions of orthopaedic prostheses made of bioinert materials have been implanted with excellent fifteen-year survivability of 75–85%. Improved metal alloys, special polymers and medical grade ceramics are the basis for this success, which has enhanced the quality of life for millions of patients.^{1–8} However, an increasing percentage of our ageing population requires greater than thirty-year survivability of devices.^{3,9} It is proposed that to satisfy this growing need for very long term orthopaedic repair that a paradigm shift is needed; a shift from emphasis on *replacement* of tissues to *regeneration* of tissues.⁸ Such a shift from a materials-and-mechanics approach to biological based tissue repair requires an increase in understanding and utilisation of molecular biology. A new biological-orientated alternative based upon use of bioactive materials for tissue engineering is described.

The concept of the use of Class A bioactive materials to stimulate the regeneration of bone provides the scientific foundation for creating bioactive resorbable scaffolds. Class A bioactive materials exhibit 11 reaction stages that lead to enhanced proliferation and differentiation of osteoblasts and recreation of trabecular bone architecture *in situ*. Recent results presented below show that the effects of microchemical gradients on the genetic activation of bone cells are related to the molecular design of hierarchical bioactive resorbable scaffolds and can be used for tissue engineering of bone constructs.

THE NEED

During the last century, orthopaedics underwent a revolution, a shift in emphasis from palliative treatment of infectious diseases of bone to interventional treatment of chronic age-related ailments.^{5,6} The evolution of stable metallic fixation devices, and the systematic development of reliable total joint prostheses were critical to this revolution in health care. The history and the current practice of these revolutionary steps are well documented¹⁻⁶ and will not be repeated here.

Devices and prostheses made from orthopaedic biomaterials ideally should survive without failure for the lifetime of the patient. The challenge is that the lifetime of patients has progressively increased during the last century⁹ and will continue to do so for many years to come. Average life expectancy is currently at 80+ years, an increase of more than 15 years since the 1960s, when Professor Sir John Charnley pioneered the use of low friction total hip replacement. There is a compound effect of increased patient lifetime on the survivability of orthopaedic prostheses: i) Many more patients need prostheses; ii) The quality of bone of the patients progressively deteriorates with age, especially for women after the menopause. The two effects are multiplicative and contribute to the

continuing decline in implant survivability with patient age described in Ref. 3.

There are two options to satisfy increasing needs for orthopaedic repair in the future: i) improve implant survivability by 10–20 years; or ii) develop alternative means of orthopaedic treatment that do not require implants, or at least delay the need for prostheses by 10–20 years. The objective of this chapter is to discuss use of tissue engineering to meet these needs.

THE BIOACTIVE ALTERNATIVE

During the last decade considerable attention has been directed towards the use of implants with bioactive fixation, where bioactive fixation is defined as interfacial bonding of an implant to tissue by means of formation of a biologically active hydroxyapatite layer on the implant surface.^{1,4,7}

An important advantage of bioactive fixation is that a bioactive bond forms at the implant-bone interface with a strength equal to or greater than bone.

Materials for clinical use can be classified into three categories: resorbable, bioactive and nearly inert materials. A bioactive material is defined as a material that elicits a specific biological response at the interface of the material, which results in a formation of a bond between the tissue and that material.⁸ The level of bioactivity of a specific material can be related to the time taken for more than 50% of the interface to bond to bone ($t_{0.5bb}$):

$$\text{Bioactivity index, } I_B = 100/t_{0.5bb} \quad (1)$$

Materials exhibiting an I_B value greater than 8 (class A), e.g. 45S5 Bioglass[®], will bond to both soft and hard tissue. Materials with an I_B value less than 8 (class B), but greater than 0, e.g. synthetic hydroxyapatite, will bond only to hard tissue.⁹ A bioactive glass is one that undergoes surface dissolution in a physiological environment in order to form a hydroxycarbonate apatite (HCA) layer.¹⁰

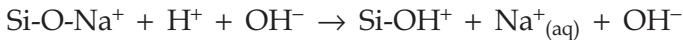
The larger the solubility of a bioactive glass, the more pronounced is the effect on bone tissue growth.¹¹

MECHANISM OF BIOACTIVITY

When a glass reacts with an aqueous solution, both chemical and structural changes occur as a function of time within the glass surface.¹² Accumulation of dissolution products causes both the chemical composition and pH of solution to change. The formation of HCA on bioactive glasses and the release of soluble silica and calcium ions to the surrounding tissue are key factors in the rapid bonding of these glasses to tissue, stimulation of tissue growth and use as tissue engineering scaffolds.¹³⁻¹⁵

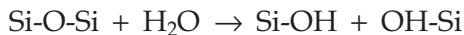
There are 11 stages in the process of complete bonding of bioactive glass to bone. Stages 1–5 are chemical; stages 6–11 are the biological response:

- i) Rapid exchange of Na^+ and Ca^{2+} with H^+ or H_3O^+ from solution (diffusion controlled with a $t^{1/2}$ dependence, causing hydrolysis of the silica groups, which creates silanols);



The pH of the solution increases as a result of H^+ ions in the solution being replaced by cations.

- ii) The cation exchange increases the hydroxyl concentration of the solution, which leads to attack of the silica glass network. Soluble silica is lost in the form of $\text{Si}(\text{OH})_4$ to the solution, resulting from the breaking of Si-O-Si bonds and the continued formation of Si-OH (silanols) at the glass solution interface:



This stage is an interface-controlled reaction with a $t^{1.0}$ dependence.

- iii) Condensation and repolymerisation of a SiO_2 -rich layer on the surface, depleted in alkalis and alkali-earth cations.

- iv) Migration of Ca^{2+} and PO_4^{3-} groups to the surface through the SiO_2 -rich layer, forming a $\text{CaO-P}_2\text{O}_5$ -rich film on top of the SiO_2 -rich layer, followed by growth of the amorphous $\text{CaO-P}_2\text{O}_5$ -rich film by incorporation of soluble calcium and phosphates from solution.
- v) Crystallisation of the amorphous $\text{CaO-P}_2\text{O}_5$ film by incorporation of OH^- and CO_3^{2-} anions from solution to form a mixed hydroxyl carbonate apatite (HCA) layer.
- vi) Adsorption and desorption of biological growth factors, in the HCA layer (continues throughout the process), to activate differentiation of stem cells.
- vii) Action of macrophages to remove debris from the site allowing cells to occupy the space.
- viii) Attachment of stem cells on the bioactive surface.
- ix) Differentiation of stem cells to form bone growing cells, osteoblasts.
- x) Generation of extracellular matrix by the osteoblasts to form bone.
- xi) Crystallisation of inorganic calcium phosphate matrix to enclose bone cells in a living composite structure.

For bone, interfacial bonding occurs because of the biological equivalence of the inorganic portion of bone and the growing HCA layer on the bioactive implant.¹⁹ For soft tissues, the collagen fibrils are chemisorbed on the porous SiO_2 -rich layer via electrostatic, ionic and/or hydrogen bonding, and HCA is precipitated and crystallised on the collagen fibre and glass surfaces.²⁰

Reaction stages one and two are responsible for the dissolution of a bioactive glass, and therefore greatly influence the rate of HCA formation. Many studies have shown that the leaching of silicon and sodium to solution is initially rapid, following a parabolic relationship with time for the first six hours of reaction, then stabilises, following a linear dependence on time.^{9,13,14,16,21-24}

For bioactive scaffolds, it is necessary to be able to control the solubility (dissolution rate) of the material. A low solubility material

is necessary if the scaffold is designed to have a long life. A controlled solubility rate is required if it is designed to aid bone formation, such as 45S5 Bioglass® powders for bone graft augmentation or rapid formation of bone *in vitro*. Therefore, a fundamental understanding of factors influencing solubility and bioreactivity is required to develop new materials for *in situ* tissue regeneration and tissue engineering.

FACTORS AFFECTING DISSOLUTION OF BIOACTIVE GLASS TO BE USED AS TISSUE ENGINEERING SCAFFOLDS

The solution parameters of initial pH, ionic concentration and temperature have a large effect on the rate of scaffold dissolution and even type of calcium phosphate precipitated.²² Ionic concentration, and therefore pH, will obviously change with time as dissolution progresses and this will in turn affect the dissolution rate. If pH rises above a critical value, cytotoxicity will occur.²⁶

Three types of media have been used during bioactive glass dissolution experiments:

- i) Tris-buffer is a simple organic buffer solution.
- ii) Simulated body fluid (SBF) is a tris buffer containing similar ion concentrations to that of human blood plasma.
- iii) α -MEM and D-MEM are culture media that contain both the inorganic and biological organic components of blood plasma.¹⁶

Zhong and Greenspan¹⁶ found that Bioglass® underwent a faster surface reaction and exhibited a larger HCA crystal size in Tris solution than it did in SBF or culture medium. However, Pereira *et al.*²⁷ found that HCA nucleated faster in SBF than in Tris, which they put down to the far higher initial P concentration (40ppm) in SBF, thus aiding HCA nucleation. A similar result was found by Tsuru *et al.*,²⁸ but they suggested that the dissolution of Ca²⁺ ions

from materials would increase the degree of supersaturation in the solution (SBF), which would already be supersaturated regarding HCA precipitation and hence make it much easier for HCA to be precipitated.

Pereira *et al.*^{22,26} found that increasing the pH or Ca^{2+} concentration of SBF containing porous silica gel–glass reduced the induction time for heterogeneous HCA formation, agreeing with the early work by Wirth and Gieskes²⁹ on anhydrous silica powders, which found the rate of silicon release increased by two orders of magnitude with each unit increase of pH. Pereira and Hench also found evidence of homogeneous HCA precipitation in SBF, containing porous silica gel–glass, when the Ca^{2+} content increased above a critical value of 300ppm.²⁶

Reaction kinetics of surface layer formation have been found to alter in culture media containing serum, due to the adsorption of serum proteins onto the surface of the bioactive glass forming a barrier to nucleation of the HCA layer.^{30–32}

GEOMETRIC EFFECTS

A change in geometry of a tissue engineering scaffold will generally mean a change in the surface area to solution volume ratio (SA/V), which will affect the dissolution rate, as the amount of surface exposed to solution for ion exchange will also change.

Elsberg *et al.*³³ reacted fibres and cylinders of melt-derived 45S5 Bioglass® in tris-buffer *in vitro*, using a SA/V ratio of 0.1cm^{-1} . Dissolution rate was found to be inversely proportional to the radius of the sample, whereas the nucleation and growth of HCA occurred earlier on surfaces with a larger radius of curvature. Release of the network modifiers, sodium and calcium, was again diffusion dependent ($t^{1/2}$). Silicon dissolution, as a function of log time, exhibited two linear regimes, rather than the parabolic/linear regimes observed in other studies. The slope of this relationship was lower during stages 1–3, formation of the silica gel layer, than for stages

4 and 5, the nucleation of the HCA layer, which implies network break-up was accelerated while the HCA layer formed.

The effect of SA/V on the dissolution of bioactive glass powders was investigated by Greenspan *et al.*,¹³ using melt-derived 45S5 Bioglass® powders of different particle size ranges, immersed in tris-buffer solution for various periods of time. In general, the higher SA/V ratios had a faster rate of pH increase and a higher final pH when compared to the lower SA/V ratios. At high SA/V ratio, the calcium phosphate layer formed rapidly but remained thin over time. At lower SA/V ratios, the calcium phosphate layers grew more slowly, but the final thicknesses after 20 hours were greater than at high SA/V ratio. The thin layer at high SA/V ratio may be due to the layer inhibiting ion exchange at the silica-gel layer surface. Therefore, a higher SA/V favours initial dissolution, yielding a faster initial calcium phosphate layer formation. When the SA/V was held constant, the rate of formation of the HCA layer was much slower for smaller particles, which was attributed to physical differences such as radius of curvature, mass to volume ratio and test solution. There appears to be a trade-off between rapid dissolution at small particle sizes (and therefore better resorbability) and thickness of HCA formation at larger particle sizes. These results are important for processing tissue engineering scaffolds incorporating bioactive powders, fibres, meshes or foams.

Wilson and Noletti³⁴ found that particles of 100µm in diameter were either resorbed or phagocytosed by macrophages *in vivo*, while larger particles stimulated bone growth. This could be a problem for using fine particles, meshes or foams in scaffolds in order to attain rapid dissolution and high bioactivity *in vivo* and requires further investigation. Schepers *et al.*³⁵ also found that glass particles of less than 300µm in diameter are fully resorbed *in vivo*. Thus it may be necessary to use a range of particle sizes, fibre diameters or foam textures to produce the necessary gradients of dissolution products to enhance bone formation *in situ* or in tissue engineering. This is presently being done, as described in recent publications.^{36,37}

COMPOSITION EFFECTS OF BIOACTIVE GLASS SCAFFOLDS

Until the late 1980s, bioactive glasses were generally melt-derived, with the majority of research aimed at the 45S5 Bioglass® composition (46.1% SiO₂, 24.4% NaO, 26.9% CaO and 2.6% P₂O₅, in mol%) and apatite-wollastanite (A/W) glass-ceramics.³⁸ The rapid rate of HCA formation exhibited by melt-derived bioactive glasses was attributed to the presence of Na₂O, or other alkali cations in the glass composition, which increased the solution pH at the implant-tissue interface as dissolution progressed.¹⁷ Adding multivalent cations, such as alumina, stabilise the glass structure by eliminating non-bridging oxygen²⁵ and also retard HCA formation, but slight deviations in composition can radically alter the dissolution kinetics or even basic mechanisms of bonding.^{10,14,39}

Hill found that increasing the SiO₂ content of the glass linearly decreased the glass transition temperature, the peak crystallisation temperature, the oxygen density in the glass, the glass density and increased the thermal expansion coefficient. He therefore concluded that Na₂O was a network disruptor and actually decreased the bioactivity of a glass.⁴⁰

It is widely accepted that increasing silica content of melt-derived glass decreases dissolution rates by reducing the availability of modifier ions such as Ca²⁺ and HPO⁴⁻ ions to the solution and the inhibiting development of a silica-gel layer on the surface.^{25,27} The result is the reduction and eventual elimination of the bioactivity of the melt-derived bioactive glasses as the silica content approaches 60%. No melt-derived glasses with more than 60mol% silica are bioactive. In order to obtain bioactivity for silica levels higher than 60mol%, the sol-gel process must be employed, which is a novel processing technique for the synthesis of tertiary bioactive glasses.

SOL-GEL-DERIVED BIOACTIVE GLASSES

The recognition that the silica gel layer plays a role in HCA nucleation and crystallisation led to the development of the bioactive three component $\text{CaO-P}_2\text{O}_5\text{-SiO}_2$ sol-gel-derived glasses by Li, Clark and Hench,²⁷ thus dispelling the theory that Na_2O was the active component of the bioactive glass.

A sol is a dispersion of colloidal particles in a liquid. Colloids are solid particles with diameters 1–100nm. A gel is an interconnected, rigid network with pores of submicrometer dimensions and polymeric chains whose average length is greater than 1 μm . Details of sol-gel processing are given in Refs. 38–43.

There are several advantages of a sol-gel-derived glass over a melt-derived glass which are important for making tissue engineering scaffolds. Sol-gel-derived glasses have:

- i) Lower processing temperatures (600–700°C).
- ii) The potential of improved purity, required for optimal bioactivity due to low processing temperatures and high silica and low alkali content.
- iii) Improved homogeneity.
- iv) Wider compositions can be used (up to 90mol% SiO_2) while maintaining bioactivity.
- v) Better control of bioactivity by changing composition or microstructure.
- vi) Structural variation can be produced without compositional changes by control of hydrolysis and polycondensation reactions during synthesis.
- vii) A greater ease of powder production.^{17,38}
- viii) A higher bioactivity.^{14,15,26,27,43,44}
- ix) Interconnected nanometer scale porosity that can be varied to control dissolution kinetics or be impregnated with biologically active phases such as growth factors.
- x) Can be foamed to provide interconnected pores of 10–200 μm , mimicking the architecture of trabecular bone.

The mechanism for dissolution and HCA formation on bioactive gel-glasses follows most of the same 11 stages as those for melt-derived glasses as listed above. The following sections compare dissolution characteristics of sol-gel-derived glasses with the melt-derived glasses described above.

Li *et al.*⁴⁵ found that, for melt-derived glasses, as the SiO₂ composition increased in CaO-P₂O₅-SiO₂ gel-glasses, the rate of HCA formation decreased. However, bioactivity of the ternary system continued up to 90mol% silica. This is due to the high concentration of nucleating sites in gel-glasses. The surface area of gel-glasses has been found to increase with silica content,^{27,46} which also enhances bioactivity.

In vitro studies by Greenspan *et al.*^{14,15} found more rapid dissolution and faster HCA layer formation for sol-gel derived 58S gel-glasses (60% SiO₂, 36% CaO and 4% P₂O₅, in mol%) (one hour) compared with 45S5 Bioglass[®] (six hours). This result was supported by *in vivo* experiments, in which after 12 weeks nearly all the silica was depleted from the 58S gel-glass and a calcium phosphate residue was observed. The 45S5 implant exhibited a loss of sodium ions, but had a lower rate of silica dissolution and a thicker HCA layer. Silica was lost at a similar rapid rate for both materials for the first six hours. After that time, the silica dissolution rate from the 58S gel-glass was more rapid than from the 45S5 glass. It took four days longer for the silica release rate from the 58S to stabilise, compared to that for the 45S5 melt-glass. Similar results were found by Hench *et al.*⁴³ *in vitro* for 58S and 45S5 particles (300–710µm), reacted in SBF but the *in vivo* results by Greenspan *et al.*^{14,15} showed no difference in bone formation between the two glasses after 12 weeks. In contrast, Chou *et al.*⁴⁷ have shown enhanced bone formation after four weeks for *in vivo* sites treated with sol-gel derived materials compared to those treated with melt-derived glasses. The greater surface area provided by the mesoporous 58S gel-glasses allowed prolonged ion exchange and more rapid dissolution as bone formed.

Therefore, the increased rate of HCA formation and higher index of bioactivity for the sol–gel-derived glasses is attributed to more release of soluble silica that nucleates HCA crystals in the nanometer-sized pores of the gel glass.¹⁴

TEXTURAL EFFECTS

In addition to composition, the nanometer scale texture of sol–gel-derived scaffolds is an important class of variables in the behaviour of these materials. The unique interconnected mesoporous structure (pores with diameter between 2 and 50nm) of sol–gel-derived glasses, which increases the SA/V ratio compared to that of melt-derived glasses, has been found to be the critical factor in enhancing the dissolution rate and rate of HCA formation. *In vitro* studies^{14,26} showed that the dissolution rate of 58S gel–glass increased as porosity and pore volume increased. Pore sizes greater than 2nm were required to achieve rapid kinetics. An increased SA/V ratio increases the surface exposed to the solution, improving ion exchange (stage 1) and a greater release of soluble silica (stage 2) that is required to form a porous silica rich layer. The nanometer-sized pores of the gel glass act as initiation sites for HCA crystal nucleation.^{26,43} The superposition of surface potentials⁴⁴ inside the pores increases the ionic concentration and degree of supersaturation of the Ca and P ions. Thus precipitation of HCA is more likely to occur first inside the pores. The rate of nucleation is then controlled by the diffusion of ions into the pores.

The porous structure extends the silica composition range of bioactive glasses from 60 to 90mol%. Although the gel-glass network break up (stage 2) is more difficult as silica content increases, the increase in SA/V ratio means that ion exchange (stage 1) is enhanced. Thus, a high concentration of Ca^{2+} and HPO_4^{4-} ions is released to the solution as pore volume increases,^{14,26} so that a silica-gel layer can develop very rapidly on the surface of the glass.^{27,45}

Sol-gel derived glasses also exhibit significant bioresorbability when their pores reach a certain size.³³ Bioresorption is defined as the resorption of a material *in vivo*, due to the action of osteoclasts, which in this case is attributed to the interconnected pore network, high surface areas and low particle density.⁴³ Although it is difficult to control resorbability by changing composition, controlling the pore texture significantly influences degradability.^{14,22} Biodegradation is mainly governed by the crystal structure, grain size, microporosity, neck geometry and crystallinity of the material.⁴⁸

Initially, it was thought that Ca^{2+} ion dissolution and resulting silanol formation on the surface were mandatory for a material to form an HCA layer.²⁸ However, Pereira and Hench²⁶ conclude that, as hydroxyl coverage is independent of textural characteristics, the concentration of silanol groups on the silica surface does not control the rate of HCA formation. There is no evidence to show that silanols are a requisite for HCA formation, but their involvement cannot be discounted.

Both a negative surface charge and a porous substrate are required for HCA formation.^{16,26} Silica presents a negative surface charge at physiological pH, which leads to the formation of an electrical double layer with an increased number of cations at the interface.⁴⁴ This provides evidence that a porous silica layer is required for an HCA layer to form. Hence, an HCA layer can be formed on a porous pure silica gel in a solution containing Ca^{2+} and HPO_4^{4-} ions.²²

CELLULAR FEATURES OF CLASS A BIOACTIVE MATERIALS

An important feature of Class A bioactive particulates is that they are osteoproliferative as well as osteoconductive. In contrast, Class B bioactive materials exhibit only *osteoconductivity*, defined as the characteristic of bone growth and bonding along a surface. Dense

synthetic hydroxyapatite (HA) ceramic implants exhibit Class B bioactivity. *Osteoproduction* occurs when bone proliferates on the particulate surfaces of a mass due to enhanced osteoblast activity. Enhanced proliferation *and* differentiation of osteoprogenitor cells, stimulated by slow resorption of the Class A bioactive particles, are responsible for osteoproduction.

The biological response to bioactive gel-glasses made from the CaO-P₂O₅-SiO₂ system provides evidence that bone regeneration is feasible. Biological molecules can exchange with hydrated layers inside the pores of gel-glasses and maintain their conformation and biological activity.^{7,42,53,57} Many enzymes remain active within a hydrated gel matrix, and in some cases exhibit enhanced activity. Such hierarchical structures and behaviour go far beyond historically important bioinert orthopaedic materials such as PMMA, ultrahigh molecular weight polyethylene, stainless steel, Co-Cr and Ti-alloys towards matching the ultrastructure and molecular chemistry of bone.

Evidence of the regenerative capacity of bioactive gel-glasses is based on comparison of the rates of proliferation of trabecular bone in a rabbit femoral defect model.⁵⁹ Melt-derived Class A 45S5 bioactive glass particles exhibit substantially greater rates of trabecular bone growth and a greater final quantity of bone than Class B synthetic HA ceramic or bioactive glass-ceramic particles. The restored trabecular bone has a morphological structure equivalent to the normal host bone after six weeks; however, the regenerated bone still contains some of the larger (>90 micrometers) bioactive glass particles.^{59,60} Recent studies show that the use of bioactive gel-glass particles in the same animal model produces an even faster rate of trabecular bone regeneration with no residual gel-glass particles of either the 58S or 77S composition.⁶⁰ The gel-glass particles resorb more rapidly during proliferation of trabecular bone. The mechanical quality of the regenerated bone appears to be equivalent to that of the control sites.⁶⁴ Thus, the criteria of a regenerative allograft appear to have been met. Our

challenge for the future is to extend these findings to studies in compromised bones, with osteopenia and osteoporosis, to apply the concept to humans with ageing bones and degenerative joint disease and to use the results to design the 3D architectures required for engineering of tissues.

GENETIC CONTROL BY BIOACTIVE MATERIALS

We have now discovered the genes involved in phenotype expression and bone and joint morphogenesis, and thus are on the way towards learning the correct combination of extracellular and intracellular chemical concentration gradients, cellular attachment complexes and other stimuli required to activate tissue regeneration *in situ* and in tissue engineering constructs. Professor Julia Polak's group at the Imperial College Tissue Engineering Centre has recently shown that six families of genes are up-regulated and down-regulated by bioactive glass extracts during proliferation and differentiation of primary human osteoblasts *in vitro*.⁶¹⁻⁶³ These findings should make it possible to design a new generation of bioactive materials for regeneration and tissue engineering of bone. The significant new finding is that low levels of dissolution of the bioactive glass particles in the physiological environment exert a genetic control over osteoblast cell cycle and rapid expression of genes that regulate osteogenesis and the production of growth factors.⁶¹⁻⁶³

Xynos *et al.* have shown that within 48 hours, a group of genes was activated including genes encoding nuclear transcription factors and potent growth factors. These results were obtained using cultures of human osteoblasts, obtained from excised femoral heads of patients (50–70 years) undergoing total hip arthroplasty.⁶¹

In particular, insulin-like growth factor (IGF) II, IGF-binding proteins and proteases that cleave IGF-II from their binding proteins were identified.⁶³ The activation of numerous early response genes and synthesis of growth factors was shown to modulate the cell

cycle response of osteoblasts to the bioactive glasses and their ionic dissolution products. These results indicate that bioactive glasses enhance osteogenesis through a direct control over genes that regulate cell cycle induction and progression. However, these molecular biology results also confirm that the osteoprogenitor cells must be in a chemical environment suitable for passing checkpoints in the cell cycle towards the synthesis and mitosis phases. Only a select number of cells from a population are capable of dividing and becoming mature osteoblasts. The others are switched into apoptosis. The number of progenitor cells capable of being stimulated by a bioactive medium decreases as a patient ages. These findings may account for the time delay in formation of new bone in augmented sites.

Clinical application of the use of a regenerative biomaterial in orthopaedics is beginning. In 1993, particulate bioactive glass, 45S5 Bioglass[®] was cleared in the USA for clinical use as a bone graft material for the repair of periodontal osseous defects. Since that time, numerous oral and maxillofacial clinical studies have been conducted to expand the material indication. More than 2,000,000 reconstructive surgeries in the jaw have been performed with the material. The same material has been used by several orthopaedic surgeons to fill a variety of osseous defects and for clinical use in orthopaedics, such as NovaBone[®], which is now approved for clinical use in Europe.⁶⁴

CONCLUSIONS

During the last century, a revolution in orthopaedics occurred which has led to a remarkably improved quality of life for millions of aged patients. Specially developed biomaterials were a critical component of this revolution. However, high rates of survivability of prostheses appear to be limited to approximately 20 years. Thus, it is concluded that a shift in emphasis from replacement of tissues to a new concept of regeneration and cellular engineering of tissues should

be the research emphasis for orthopaedic materials in the years ahead. The emphasis should be on the use of materials to activate the body's own repair mechanisms, i.e. regenerative allografts and tissue engineered constructs. This concept will combine the understanding of osteogenesis and chondrogenesis at a molecular level with the design of a new generation of bioactive materials that stimulate genes that activate the proliferation and differentiation of osteoprogenitor cells and enhance rapid formation of extracellular matrix and growth of new bone *in situ*. The economic and personal benefits of *in situ* regenerative repair of the skeleton for younger patients will be profound.

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64. Details of the clinical cases are available from US Biomaterials Inc., Alachua, FL, USA, 32615.