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The story of Bioglass®

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Abstract Historically the function of biomaterials has been to *replace* diseased or damaged tissues. First generation biomaterials were selected to be as bio-inert as possible and thereby minimize formation of scar tissue at the interface with host tissues. Bioactive glasses were discovered in 1969 and provided for the first time an alternative; second generation, interfacial bonding of an implant with host tissues. Tissue regeneration and repair using the gene activation properties of Bioglass® provide a third generation of biomaterials. This article reviews the 40 year history of the development of bioactive glasses, with emphasis on the first composition, 45S5 Bioglass®, that has been in clinical use since 1985. The steps of discovery, characterization, *in vivo* and *in vitro* evaluation, clinical studies and product development are summarized along with the technology transfer processes.

1 Prologue: Learning that research is fun

It is a great honour and pleasure to share memories of the past and a vision of the future of biomaterials with friends and colleagues at this symposium. Seeing many former students who have all done so well in their careers is a special treat. My goal in this paper is to recount some of the key events that make up the story of Bioglass®. The story herein will be brief and the references limited. For a more comprehensive version see the recent review by myself, June Wilson and Dave Greenspan [1] which summarizes more than 500 papers published about bioactive glasses and glass-ceramics. For an even more detailed historical account see the book "Breaking the Biocompatibility Barrier: The development and application of bioactive materials" by L.L. Hench to be published by Imperial College Press and World Scientific Publishing in 2006 [2].

My love of research, eventually leading to the discovery of Bioglass®, began at The Ohio State University (OSU) in 1957–58 when we learned in our ceramic engineering laboratory classes how to make formulations of glasses, glazes, enamels and whitewares. I am grateful that Professors J.O. Everhart, Ralston Russell, Jr., Maynard King, and Henry Blau allowed us to do more than just follow traditional recipies. My first exposure to making a new ceramic material occurred in 1959 while working as a summer engineer intern at the General Electric Jet Engine plant in Evandale, Ohio, a suburb of Cinncinnati. My job was to operate a chemical vapour deposition reactor designed to produce a coating of dense alumina (Al_2O_3) on the exterior and interior of uranium dioxide (UO_2) fuel tubes. This was new key technology required to protect the core of a nuclear powered ramjet engine being developed by the U.S. Atomic Energy Commison (AEC). I learned during that summer that processing of ceramics was far more complex than "Mix'em and fire'em". The results of the chemical reactor were highly variable. Identifying and helping to modify the process to produce a reliable and uniform dense coating over an array of tubes at a production rate was enormously satisfying, even though the nuclear jet engine never got off the ground.

This summer experience led to even more fun and a greater challenge in 1961 when I had the opportunity as a recent ceramic engineering graduate of OSU to work on the first atomic rocket engine being developed at the Lawrence Livermore Laboratory (LLL) in Livermore, California. My assignment was to determine the effect of chemical addiditives

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on the sintering of beryllium oxide (BeO) to be used as the neutron moderator in the nuclear rocket engine. My supervisor, Dr. Ray Cooperstein, gave me considerable freedom, within the limits of working with one of the most toxic materials known to man, to design and conduct the experiments. Our results were presented at the annual meeting of the American Ceramic Society in 1962, my first paper!

At that time sintering theory assumed that any oxide additive of a higher or lower valence metal would enhance rates of densificiation by increasing the concentration of vacancies which in turn would enhance both bulk and grain boundary diffusion and sintering. However, a key paper on the effects of additives on the sintering of MgO by Nelson and Cutler showed an exception to the theory; compacts of MgO and Cr_2O_3 did not densify! Why? Discussions with Drs. J. Birch Holt and Ralph Condit, both experts at LLL in diffusion as well as sintering, revealed that this anomaly was a mystery. "A good topic for an MSc thesis", I concluded. Professor Russell agreed to supervise my graduate studies at OSU to pursue this topic, made possible by an Owens-Illinois Fellowship.

The research was fun and the answer was satisfying. When Cr_2O_3 is heated in air it reacts with O_2 to form CrO_3 which has a high vapour pressure, evaporates to coat the MgO grains, reacts to form a spinel ($Mg_2Cr_2O_4$), that coats the MgO grain boundaries and stops diffusion and sintering. Proving this explanation became my PhD dissertation, "Sintering and Reactions of MgO and Cr_2O_3", with the degree awarded in 1964.

However, my proposal for the O-I Fellowship was to study the molecular mechanisms involved in nucleation of the crystal phase of glass-ceramics, an important new commercial product. I chose to investigate the subject by use of dielectric relaxation spectroscopy to follow changes in the dielectric losses as mobile Li^+ cations in 33 mole% Li_2O-67 Mole% SiO_2 (Li_2O-$2SiO_2$) glasses became immobile as they were incorporated in $Li_2Si_2O_5$ (lithium disilicate) crystals.

I did not make much progress on the mechanisms of glass nucleation at OSU but continued the research after accepting a position in 1964 as Assistant Professor at the University of Florida, Gainesville, Florida in the newly created program that became the Department of Materials Science and Enginering, founded by Professor Fred Rhines. This research area became very fruitful, largely because it attracted the interest of Steve Freiman and Don Kinser, who both chose to do their PhD research with my supervision. Both were outstanding researchers and the series of papers resulting from their theses provided an insight for later investigations of the molecular behaviour of the complex SiO_2-CaO-Na_2O-P_2O_5 Bioglass® system.

My first U.S Department of Defense funded project started at the U of F in 1966 as part of a larger multi-disciplinary research program on "Unconventional Semiconductors", with Fred Lindholm, Professor of Electrical Enginering as Program Director. I chose to investigate the electronic behaviour of vanadium phosphate [V_2O_5-P_2O_5] glasses. We learned that these amorphous semiconductors exhibited fascinating properties, especially when heat treated to induce small regions of order. What was especially exciting was our discovery that these semi-conducting glass-ceramics has very high electronic conductivity that resisted radiation damage [3, 4]. This finding meant that these new materials might be useable as electrical switches in satellites that could survive high doses of high energy radiation, such as produced by solar flares or certain types of weapons.

I looked forward to talking about these radiation resistant electronic materials at an U.S. Army Materials Research Conference held at the Sagamore, New York Conference site in the summer of 1967. My opportunity to talk about them came earlier than expected. I shared a bus ride to the conference with an Army colonel who had recently returned to the US after a tour of duty in Vietnam as a supply officer with the Army Medical Corps. Colonel Klinker listened patiently to my enthusiastic description of our recent gamma ray experiments on the vanadia-phosphate semiconductors. When I paused, he asked a question that changed my life; "If you can make a material that will survive exposure to high energy radiation can you make a material that will survive exposure to the human body?"

I was perplexed by his question and asked for an explanation. He described numerous amputations he had witnessed in Vietnam. The colonel maintained that there was usually no option because the body rejected the metallic and plastic parts available to the surgeons. He said, "We can save lives but we cannot save limbs. We need new materials that will not be rejected by the body." This statement provided the incentive to attempt something new, to discover a material that would not form an interfacial layer of scar tissue but instead would form a living bond with the host tissues. Bioglass® resulted from that quest.

2 The hypothesis of Bioglass® : 1967–69

Returning to Gainesville, Florida after the conference I discussed the problem of rejection of metals and plastic implants with a friend and research assistant, Ray Splinter, who was in medical school. Ray confirmed the problem and set up a series of meetings with two orthopaedic faculty members at the U of F, Drs. Ted Greenlee and Bill Allen. Both agreed to participate in a research project if the US Army was willing to fund it. A proposal was submitted to the US Army Medical R and D Command in 1968, based upon a simple hypothesis:

"The human body rejects metallic and synthetic polymeric materials by forming scar tissue because living tissues are not

composed of such materials. Bone contains a hydrated calcium phosphate component, hydroxyapatite [HA] and therefore if a material is able to form a HA layer in vivo it may not be rejected by the body."

3 The discovery of Bioglass® : 1969–71

The US Army Medical R and D Command funded the proposal for a one year test of the hypothesis. I used the Na_2O-CaO-SiO_2 diagram in Phase Diagrams for Ceramics to design the first three compositions (Fig. 1). The glass composition of 45% SiO_2-24.5% Na_2O-24.5% CaO-6% P_2O_5 was selected to provide a large amount of CaO with some P_2O_5 in a Na_2O-SiO_2 matrix. The composition is very close to a ternary eutectic, making it easy to melt. The glass was melted, cast and made into small rectangular implants for testing in a rat femoral implant model designed by Dr. Ted Greenlee. The implants were made in the Department of Materials and inserted into the rats at the Gainesville, Florida Veterans Administration Hospital. The first tests were for six weeks. Dr. Greenlee reported at the end of the six weeks,

"These ceramic implants will not come out of the bone. They are bonded in place. I can push on them, I can shove them, I can hit them and they do not move. The controls easily slide out."

This finding was the basis for the first paper published in 1971 in the Journal of Biomedical Materials Research that summarised the *in vivo* results and the *in vitro* tests that provided an explanation for the interfacial bonding of the implant to bone [4]. The *in vitro* tests showed that the 45S5 Bioglass® composition (see Table 1) developed a hydroxyapatite layer in test solutions that did not contain calcium or phosphate ions. This rapid formation of HA *in vitro* was equivalent to

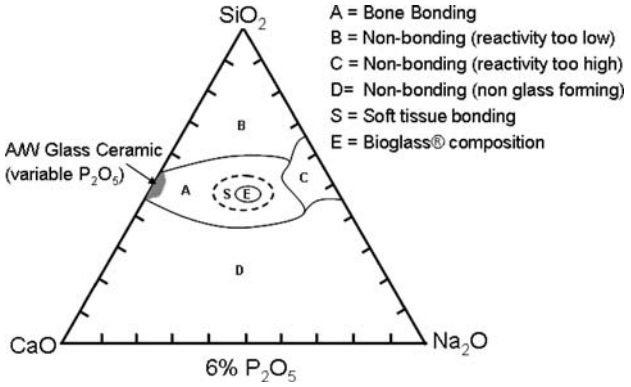

Fig. 1 Compositional diagram for bone-bonding. Note regions A, B, C, D. Region S is a region of Class A bioactivity where bioactive glasses bond to both bone and soft tissues and are gene activating

the interfacial HA crystals observed *in vivo* by Dr Greenlee's transmission electron micrographs of the bonded interface [5]. The HA crystals were bonded to layers of collagen fibrils produced at the interface by osteoblasts. The chemical bonding of the HA layer to collagen created the strongly bonded interface [4–7].

4 Bioglass®-bone bonding: 1969–78

The US Army Medical R and D Command continued funding of the project titled "An Investigation of Bonding Mechanisms at the Interface of a Prosthetic Material" for ten years. During that time a series of questions was addressed, raised by the discovery that interfacial bonding can occur between living tissues and non-living implant materials. These questions included:

(1) What is the physical, chemical and biological nature of the bond?
(2) What are the reaction mechanisms involved to form the bond?
(3) How rapidly does the bond form?
(4) What is the mechanical strength of the bond?
(5) Is the rate of bond formation, properties of the bond or bond stability influenced by composition of the implant material?
(6) Can bonding be obtained at the interface with prostheses that withstand functional loads?
(7) What is the response of other tissues to the bioactive material?

These questions were answered during the decade from 1969 to 1979 with a multi-disciplinary team of materials scientists, orthopaedic surgeons, dental researchers, biomechanics experts and biologists at the University of Florida. Drs. Homer Paschall and William Petty, Professosrs in the Department of Orthopaedics, provided guidance of the bone biology studies and Dr. Harold Stanley, Professor of Oral Medicine in the College of Dentistry, led the dental implant studies. Numerous PhD students completed theses answering the questions, usually having to develop new analytical methods to produce results. The first of such students, now Professor in the College of Dentistry at the University of Florida, was A.E. [Buddy] Clark. Pioneering papers came from his studies. A key review article that summarizes the answers to the questions listed above was published in 1982. It is ref. [8]; "Adhesion to Bone" by L.L. Hench and A.E. Clark.

The paper documents in Part A the time sequence of bonding of Bioglass® in rat femur and tibia. In Part B, bonding of Bioglass® implants to the femur in canine and monkey bones is summarized. Part C reviews the data of bonding

of mandibular and maxillar bone of primates and swine to Bioglass® implants. All species exhibited stable bone bonded implants.

5 Bioglass®-bone bond strength

One of the most difficult topics studied in the first decade of Bioglass® experiments was determining the strength of the bond to bone. The experiments were designed by Professor George Piotrowski, Head of the Biomechanics group in the Department of Mechanical Engineering and Professors Bill Allen and Bill Petty in the Department of Orthopaedics. Eight different bio-mechanical test models were developed. A quantitative evaluation of interfacial shear strength in rat and monkey models showed that the strength of the interfacial bond between Bioglass® and cortical bone was equal to or greater than the strength of the host bone [9–11]. Weinstein et al. published a key paper describing the biomechanics of the bonded interface [12].

6 Bioglass® surface reactions

Bone bonding occurs as a result of a rapid sequence of chemcial reactions on the surface of the implant when inserted into living tissues. Doctoral students D. F. Sanders, D. E. Clark, E. C. Ethridge, F. Ohuichi, M. Ogino, D.C. Greenspan and C. G. Pantano developed new techniques that made it possible to determine the kinetics of the surface reactions with great precision. Guy LaTorre used newly developed Fourier Transform Infrared Reflection Analysis techniques to quantify all five stages of the surface reactions. A review of the findings up to 1981 was published as reference [13]. Later studies were incorporated in extensive reviews [14–16].

The first five reaction stages lead to rapid release of soluble ionic species and formation of a high surface area hydrated silica and polycrystalline hydroxy carbonate apatite (HCA) bi-layer on the glass surface. The reaction layers enhance adsorption and desorption of growth factors (Stage 6) and influence the length of time macrophages are required to prepare the implant site for tissue repair (Stage 7) and the attachment (Stage 8) and synchronised proliferation and differentiation of osteoblasts (Stage 9). Mineralization of the matrix (Stage 10) follows soon thereafter and mature osteocytes, encased in a collagen-HCA matrix, are the final product by 6–12 days *in vitro* and *in vivo.*

7 Confirmation of bone bonding

Confirmation of Bioglass® bone bonding was achieved in 1976 by Professor Peter Griss in Heidlberg, Germany. Bioglass® coated alumina implants developed by Dr. David Greenspan in his PhD thesis [17] were tested as load bearing protheses in sheep. The results showed bone bonding but the coatings were not stable [18].

In 1977 a bioactive glass-ceramic based upon the 45S5 Bioglass® formula with small additions of K_2O and MgO, trademarked Ceravital®, was implanted in animal models by Professor Ulrich Gross and colleagues at the Free University of Berlin. They found that the glass-ceramic bonded to bone with a mechanically strong interface [19–20]. Additions of multi-valent cations, such as Ti and Ta, to the glass composition prevented bonding. A histological analyses of the bone bonded interfaces and mechanisms of bioactive bonding were reviewed by Gross et al. [20]. Clinical use of this bioactive material was limited due to instability of the crystal phase boundaries in the glass-ceramic.

The most important modification of bioactive glasses was the development of A/W (apatite/wollastonite) bioactive glass-ceramic by Professors T. Yamamuro and T. Kokubo and colleagues at Kyoto University, Kyoto, Japan [21–24]. A unique processing method produced a very fine-grained glass-ceramic composed of very small apatite (A) and wollastonite ($W = CaSiO_3$) crystals bonded by a bioactive glass interface [21]. Mechanical strength, toughness and stability of AW glass-ceramics (AW-GC) in physiological environments are excellent. Bone bonded to A/W-GC implants with high interfacial bond strengths [22]. Numerous animal tests led to approval to use the AW-GC material in orthopaedic applications in Japan with particular success in vertebral replacement and spinal repair, special interests of Prof. Yamamuro [23, 24]. At this symposium he reported clinical success in more than 3,000 cases of vertebral prostheeis, 12,000 cases of laminoplasty and 20,000 cases of iliac crest prostheses using AW-GC.

A third group that confirmed bonding and clinical effectiveness of bioactive glasses was led by Dr. Orjan Andersson and Professors Kai Karlsson and Antti Yli-Urpo at Abo Academy and University of Turku, Finland. Glasses modified from the 45S5 composiitonal range were designed by Karlsson and Andersson in the 1980s and implanted in animal models [25, 26]. Compositions within boundaries similar to those in Fig. 1 bonded to bone; glasses outside the bioactive boundary did not bond. Clinical use in head and neck surgical repair has been successful for many years, as reviewed in this symposium.

8 Bioglass® bone-bonding composition boundary

The compositional range for bonding of bone to bioactive glasses and glass-ceramics is illustrated as region A in Fig. 1. The boundaries are kinetic boundaries not phase equilibrium boundaries. The glass structure and reaction mechanisms responsible for the compositional boundaries are reviewed in

refs. [15, 16]. Glasses with the highest level of bioactivity and rapid bone bonding lie in the middle of the Na_2O-CaO-SiO_2 diagram (region E); all compositions contain a constant 6 weight percent of P_2O_5. Compositions that exhibit slower rates of bonding lie between 52 to 60% by weight of SiO_2 in the glass. Compositions with greater than 60% SiO_2 (region B) do not bond and are bio-inert. Increasing the surface area of the glass by making a particulate or a nanoporous sol-gel derived glass extends the bone bonding compositions to higher percentages of SiO_2 in the glass. Adding multivalent cations, such as Al^{3+}, Ti^{4+} or Ta^{5+} to the glass shrinks the bone bonding boundary [17, 19, 27].

9 Soft tissue bonding, toxicology and biocompatibility

Until 1981 it was assumed that only calcified tissues would form a bond to bioactive materials. A paper by Dr. June Wilson et al. "Toxicology and Biocompatibility of Bioglass®" [28] was the first to show that soft connective tissues could also form a bond to 45S5 Bioglass® if the interface was immobile. This paper also documented more than twenty *in vitro* and *in vivo* tests that established the safety of use of particulate forms of Bioglass® as well as bulk implants. This compendium of data provided the basis for ethical committee approval of the use of Bioglass® in clinical trials at the University of Florida and Guy's Hospital in London.

Dr. Wilson continued investigation of the interfacial interaction of soft tissues and established, in a key paper with David Nolleti, the compositional dependence of the bonding of bioactive glasses to soft tissues [29]. Only glass compositions with rapid reaction rates form a soft tissue bond. These glasses are restricted to the compositions in the region S in Fig. 1. When the glass composition exceeds 52% by weight of SiO_2 the glass will bond to bone but not to soft tissues. This finding provided the basis for clinical use of Bioglass® in ossicular replacement and also for implants to maintain the alveolar ridge of endentulous patients.

10 Classes of bioactivity

Bioactive materials used for either tissue replacement or for tissue regeneration must possess controlled chemical release kinetics that synchronise with the sequence of cellular changes occurring in wound repair [16, 30, 31]. If dissolution rates are too rapid the ionic concentrations are too high to be effective. If the rates are too slow the concentrations are too low to stimulate cellular proliferation and differentiation. Large differences in rates of *in vivo* bone regeneration and extent of bone repair, documented in papers by Oonishi et al. [32, 33] and Wheeler et al. [34, 35] indicate that there are two classes of bioactive materials. Class A bioactivity leads to *both* osteoconduction and osteoproduction [16, 31, 36] as a consequence of rapid reactions on the bioactive glass surface. The surface reactions involve ionic dissolution of critical concentrations of soluble Si, Ca, P and Na ions that give rise to both intracellular and extracellular responses at the interface of the glass with its physiological environment. Class B bioactivity occurs when only osteoconduction is present; i.e. bone migration along an interface, due to slower surface reactions, minimal ionic release and only extracellular responses occur at the interface [16, 32]. Differences between Class A and B bioactive materials are summarised in reference [15, 16].

11 Regulatory classification of bioglass® medical devices

By the mid 1980's sufficient animal data had been accumulated that safety of use of bioactive glasses as prostheses seemed assured [28]. Ethical permission was obtained from the J. Hillis Miller Health Center at the Univeristy of Florida to commence clinical trials of middle ear prostheses in the College of Medicine under the direction of ENT surgeon, Professor Gerry Merwin. Trials of endosseous ridge maintenance implants for preservation of the edentulous alveolar ridge began in the College of Dentistry under direction of Dr. Harold Stanley, Professor of Oral Medicine and Drs. A.E. Clark and Matt Hall. Successful results from these trials led to application for regulatory approval of commercial use of Bioglass® protheses.

The classification of medical devices for regulatory purposes is related to the inherent risks of the device in question and different regulatory control mechanisms are assigned to each class. To date, all Bioglass® devices placed into use in the United States have been cleared via the 510[k] process either as Class II or Class III devices. Thus, the Bioglass® devices have been able to demonstrate equivalence in safety and efficacy to devices that were already in commerce prior to 1976. The significant base of scientific studies conducted using Bioglass®, along with the required biocompatibility and toxicology studies required by the regulatory bodies have provided a strong basis for establishing the safety of Bioglass® devices placed into commerce. Quality assurance tests have been established to ensure safety and efficacy [37]. The University of Florida was granted approval of the trademark classification Bioglass® to distinguish the material from other bioactive glass and glass-ceramic products being developed world wide.

12 First clinical products: Tissue replacement

The first Bioglass® device cleared for marketing in the United States was a device used to treat conductive hearing loss by replacing the bones of the middle ear. The device was called the "Bioglass® Ossicular Reconstruction Prosthesis", and tradenamed 'MEP®'. The device was cleared via the 510[k] process in January 1985. It was a solid, cast Bioglass® structure that acted to conduct sound from the tympanic membrane to the cochlea. The advantage of the MEP® over other devices in use at the time was its ability to bond with soft tissue (tympanic membrane) as well as bone tissue. Clinical studies [38, 39] showed that the MEP® outperformed other bioceramic and metal prostheses. A modification of the MEP design was made to improve handling in the surgery and it is used clinically with the trademark name of the DOUEK MED, after Mr Ellis Douek, Professor of ENT surgery at Guy's Hospital, London who pioneered the design and tested the improved device.

Other uses in head and neck surgery of bioactive glasses are described in more than 20 citations in ref. [1].

The second Bioglass® device to be placed into the market was the Endosseous Ridge Maintainence Implant [ERMI®], which was cleared via the 510[k] process, in November, 1988. The device was designed to support labial and lingual plates in natural tooth roots and to provide a more stable ridge for denture construction following tooth extraction. The devices were simple cones of 45S5 Bioglass® that were placed into fresh tooth extraction sites. They bonded to the bone tissue and proved to be extremely stable, with much lower failure rates than other materials that had been used for that same purpose. Numerous clinical studies have been published as cited in ref. [1] and the data is on file at NovaBone Products, LLC., Alachua, Florida. A five year study quantified the substantial improvements in clinical success over Class B bioactive HA tooth root implants [40, 41].

13 Bioactive composites

The limited mechanical strength and low toughness of bioactive glasses has prevented their use as load bearing devices. Combining the mechanical properties of metals or polymers with a bioactive phase of either particles or fibres to produce a bioactive composite with optimised properties has long been a goal. As early as 1973 the author described the theoretical basis for achieving a bioactive composite material that incorporated the recently discovered bone bonding characteristics of 45S5 Bioglass® [42]. The only clinically successful bioactive composite to date is that developed by Professor William Bonfield and colleagues at the Interdisciplinary Research Centre in Biomedical Materials, University of London. The material is composed of bioactive HA particles uniformly dispensed in a dense polyethylene matrix. The composite is widely used clinically as "HAPEX" for middle ear reconstruction. The unique combination of rapid shaping in the operating theatre combined with bioactivity makes it "surgeon friendly." Details are reviewed by Rea and Bonfield [43]. These novel materials are compared with many other composites in refs. [44, 45].

14 Third-generation biomaterials

The concepts of bioactive materials and resorbable biomaterials have converged into a new, third generation of biomaterials; bioactive materials are being made resorbable and resorbable polymers are being made bioactive [46]. Molecular modifications of resorbable polymers and bioactive composite systems elicit specific interactions with cell integrins and thereby direct cell proliferation, differentiation, and extracellular matrix production and organisation. Third-generation bioactive glasses, composites, hybrid materials and macroporous foams are being designed to activate genes that stimulate regeneration of living tissues.

Two alternative routes of repair are now available with the use of third generation, molecularly tailored biomaterials.

Tissue engineering. Progenitor cells are seeded onto biologically active resorbable scaffolds. The cells grow outside the body and become differentiated and mimic naturally occurring tissues. These tissue-engineered constructs are then implanted into patients to replace diseased or damaged tissues. With time the scaffolds are resorbed and replaced by host tissues that include a viable blood supply and nerves. The living tissue-engineered constructs adapt to the physiological environment and should provide long-lasting repair. Clinical applications include repair of articular cartilage, skin, and the vascular system, although stability of the repaired tissues needs improvement.

In situ tissue regeneration. This approach involves the use of biomaterials in the form of powders, solutions, or doped microparticles to stimulate local tissue repair. Bioactive materials release chemicals in the form of ionic dissolution products, or growth factors such as bone morphogenic protein (BMP), at controlled rates, by diffusion or network breakdown, that activate the cells in contact with the stimuli. The cells produce additional growth factors that in turn stimulate multiple generations of growing cells to self-assemble into the tissues *in situ* along the biochemical and biomechanical gradients that are present. NovaBone, NovaMin and NovaThera products are all third generation bioactive glass products.

15 Bioactive reaction mechansims: Genetic control of osteoblast cell cycle

For many years it was assumed that formation of a biologically active HCA surface reaction layer was the critical requirement for bioactive behaviour [15, 16, 31, 47]. Recent studies show formation of a surface HCA layer to be a useful but not the critical stage of reaction for bone regeneration. The key phenomenon is controlled rates of release of ionic dissolution products, especially critical concentrations of soluble silica and calcia ions.

In order for new bone to form it is essential for osteoprogenitor cells to undergo mitosis. There are very few cells in the bones of older people that are capable of dividing and forming new bone. The osteoprogenitor cells that are present must receive the correct chemical stimuli from their local environment that instruct them to enter the active segments of the cell cycle [49–51]. Resting cells are in the G_0 phase (Fig. 2). Every new cell cycle begins after a cell has completed the preceding mitosis. If the local chemical environment is suitable, and following a critical period of growth in the G1 phase, the cell enters the S phase when DNA synthesis begins. The S phase eventually leads to duplication of all the chromosomes in the nucleus. Next the cell is ready to undergo mitosis with a second phase of growth termed the G2 phase. During G2 the cell prepares to undergo division and checks its replication accuracy using DNA repair enzymes. A critical increase in mass and synthesis and activation of various growth factors is necessary for the G2-M transition. Details of the feedback controls and cell cycle checkpoints are reviewed in refs. [50, 61]. If the local chemical environment does not lead to completion of the G1 phase or the G2 phase then the cell proceeds to programmed cell death, apoptosis. Bioinert materials or Class B bioactive materials do not produce the local chemical environment to enable the few osteoprogenitor cells present to pass through these cell cycle checkpoints. Only Class A bioactive materials produce rapid new bone formation *in vivo*, a process termed osteoproduction, discussed above and in ref. [36].

The time for formation of collagen on bioactive substrates*in vitro* is similar to the kinetics of collagen formation *in vivo* [15, 50–64]. The rate of forming mineralised bone nodules *in vitro* [51, 60–65] is also similar to the kinetics of bone growth *in vivo* [32, 64, 66–72]. The 3D architecture of

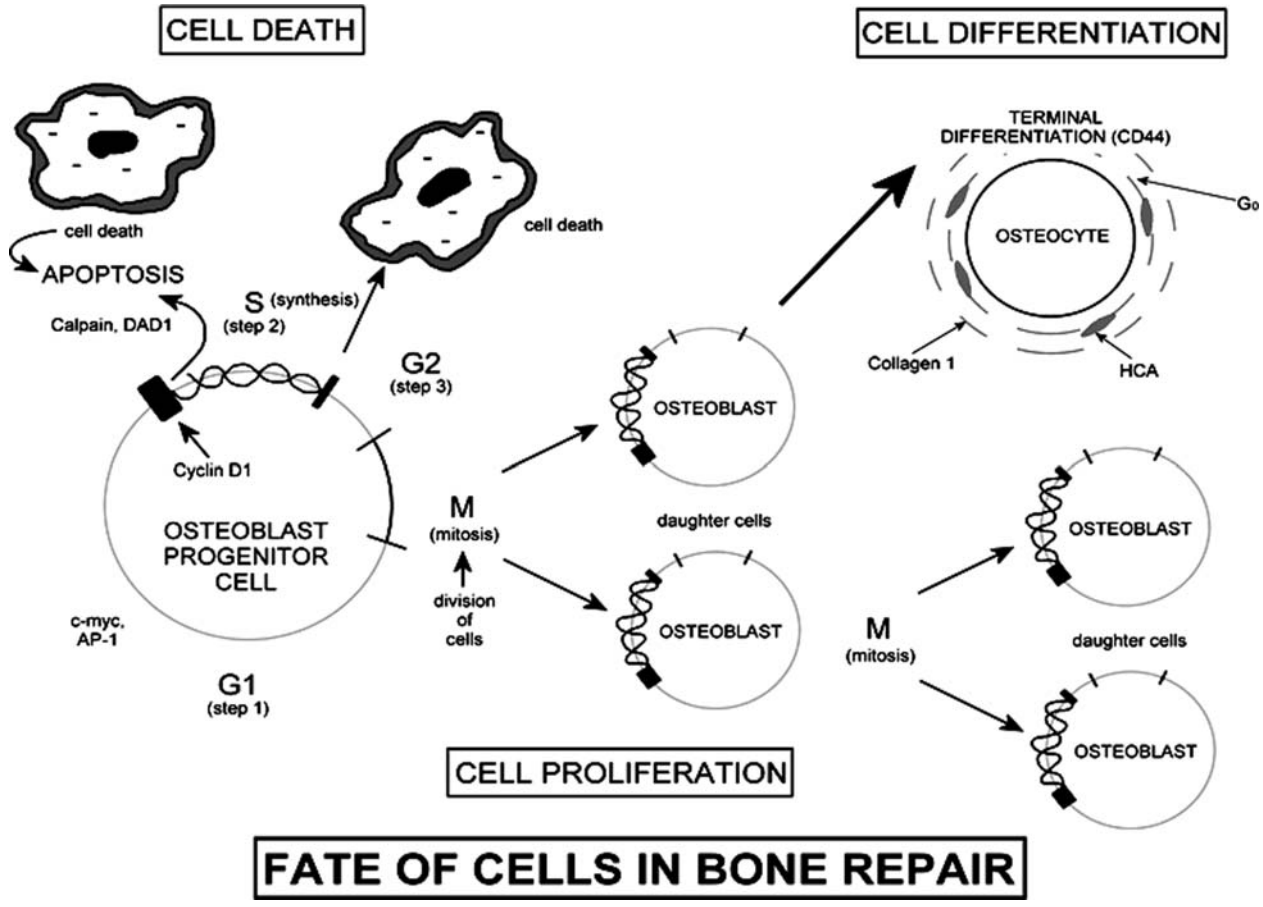

Fig. 2 Schematic of osteoblast cell cycle leading to rapid cell proliferation and differentiation when exposed to bioactive ionic dissolution products released by Bioglass 45S5

mineralized bone is created by the osteoblasts when the cells are exposed to critical concentrations of the soluble ionic constituents released from bioactive glasses. Approximately 17 to 20 ppm of soluble Si and 88 to 100 ppm of soluble Ca ions are required. The ions are provided by controlled dissolution of a bulk implant or particulate. The role of the bioactive glass is primarily to release the critical concentrations of biologically active ions at the rate needed for cell proliferation and differentiation [50–55].

The number of bone nodules growing on Class A bioactive glass substrates increases from 6 to 12 days *in vitro* and the organisation of the nodules becomes increasingly more complex with large numbers of osteocytes within the nodules [51, 52]. At day 12 there are still no bone nodules present on bioinert substrates although the osteoblast-like cells are still healthy. The cellular markers suggest that the cells growing on the inert or Class B bioactive materials, such as dense HA substrates, are not capable of forming new mineralised bone, but are more similar to the fibroblast-like cells found in scar tissues.This explains the slow rates of bone growth found by Oonishi et al. [33, 64, 66, 67] and poor mechanical properties of the tissues grown in the presence of Class B bioactive materials by Wheeler et al. [35].

Molecular biology studies by Xynos et al. in Professor Dame Julia Polak's group at Imperial College London, show that the bioactive shift of osteoblast cell cycle is under genetic control [50–55, 61–63]. Within a few hours exposure of human primary osteoblasts to the soluble chemical extracts of 45S5 Bioglass®, several families of genes are activated, including: genes encoding nuclear transcription factors and potent growth factors, especially IGF-II along with IGF binding proteins and proteases that cleave IGF-II from their binding proteins [52]. There is a 200 to 500% increase in the expression of these genes over those of the control cultures. Activation of several immediate early response genes and synthesis of growth factors is likely to modulate the cell cycle response of osteoblasts to bioactive glasses. These findings indicate that Class A bioactive glasses enhance new bone formation (osteogenesis) through a direct control over genes that regulate cell cycle induction and progression.

As discussed earlier, the cell cycle does not merely provide the framework for cell proliferation but also determines to some extent cell commitment and differentiation. Bone cells cover a broad spectrum of phenotypes that include predominately the osteoblast, a cell capable of proliferating and synthesising bone cell specific products such as Type I collagen. However, a vital cellular population in bone consists of osteocytes that are terminally differentiated osteoblasts. Osteocytes are postmitotic and not capable of cell division. They are capable of synthesising and maintaining the mineralised bone matrix wherein they reside. Thus, osteocytes represent the cell population responsible for extracellular matrix production and mineralisation, the final step in bone development and probably the most crucial one given the importance of collagen-hydroxyl carbonate apatite (HCA) bonding in determining the mechanical function of bone. Therefore, it is important to observe that the end result of the cell cycle activated by the ionic products of bioactive glass dissolution is the upregulation of numerous genes that express growth factors and cytokines and extracellular matrix components. Also, there is a 700% increase in the expression of CD44 a specific phenotypic marker of osteocytic differentiation.

Xynos et al.'s cDNA microarray analysis showed that expression of a potent osteoblast mitogenic growth factor, insulin-like growth factor II [IGF-II] was increased to 320% by exposure of the osteoblasts to the bioactive glass stimuli [52]. This is an important finding because IGF-II is the most abundant growth factor in bone and is also a known inducer of osteoblast proliferation *in vitro*. The results indicate that the ionic dissolution products of Bioglass® 45S5 may increase IGF-II availability in osteoblasts by inducing the transcription of the growth factor and its carrier protein and also by regulating the dissociation of this factor from its binding protein. The unbound IGF-II is likely to be responsible for the increase in cell proliferation observed in the cultures. Similar bioactive induction of the transcription of extracellular matrix components and their secretion and self-organisation into a mineralised matrix may be responsible for the rapid formation and growth of bone nodules and differentiation of the mature osteocyte phenotype in the presence of Class bioactive materials such as 45S5 Bioglass® and sol-gel derived bioactive gel glasses tested by Beilby et al. in refs. [62] and [63].

16 Third generation clinical products

While the Second Generation Bioglass® materials performed admirably in replacing diseased or missing hard tissue, the discoveries that Bioglass® could positively affect osteoblasts, and in fact 'stimulate' them to produce more bone tissue earlier than other synthetic biomaterials led to the concept of 'osteoproduction' and 'osteostimulation'. In order to take advantage of this property, and of the need to regenerate diseased or missing tissues, the development of third generation Bioglass® products focused on using particles rather than monolithic shapes. The products are being manufactured and sold to the clinic under the name NovaBone.

The first NovaBone® particulate material cleared for sale in the U.S. was PerioGlas®, which was cleared via the 510[k] process in December, 1993. In 1995, PerioGlas® obtained a CE Mark and marketing of the product began in Europe. The initial indication for the product was to restore bone loss

resulting from periodontal disease in infrabony defects. In 1996, additional indications for use were cleared by FDA, including use in tooth extraction sites and for alveolar ridge augmentation.

The first paper to describe potential use of 45S5 Bioglass® particulate in repair of periodontal defects was published in 1987 by Dr. June Wilson and Professor Sam Low, Department of Periodontology and colleagues at the University of Florida [73]. A detailed study of the monkey model followed in 1992 [36], and 1994 [74]. Schepers et al. reported similar findings in a different animal model [75]. Other related studies are in refs. [76–78].

During a ten-year clinical history, PerioGlas® has demonstrated excellent clinical results with virtually no adverse reactions to the product. Numerous clinical studies have demonstrated the efficacy of the product in multiple uses. See reference [1] for a listing of twenty or more clinical studies. To date, PerioGlas® is sold in over 35 countries, and the manufacturer estimates that the product has been used in nearly one million surgeries [Data on file at US Biomaterials Corporation].

Building on the successes of PerioGlas® in the market, a Bioglass® particulate for orthopedic bone grafting was introduced into the European market in 1999, under the trade name NovaBone® . Early studies by Dr. June Wilson et al. in a canine model showed effective bone regeneration with uses of 45S5 Bioglass® particulate [79]. Other animal models followed in various laboratories worldwide, as reviewed in ref. [1]. The product was cleared for general orthopaedic bone grafting in non load bearing sites in February, 2000. This material is still in the early stages of clinical use, and clinical studies with average follow-up of two years are expected to be published by the end of 2005. To date, NovaBone® is being sold in the U.S. and Europe, as well as in China and a number of other countries.

More recently, Bioglass® particulate has been used for the treatment of dentinal hypersensitivity. Tooth hypersensitivity is a problem that affects an estimated 15 to 20% of the population of the United States, and similar numbers in Europe. Tooth hypersensitivity occurs when the root portion of the tooth, which is dentin, becomes exposed around the gum line. The dentin has small openings, or tubules, that communicate with the pulp chamber. If the dentinal tubules become exposed, hot or cold or pressure can transmit the sensations to the nerves in the pulp, causing pain. The Bioglass® material used in this application is a very fine particulate that is incorporated into toothpaste, or used with an aqueous vehicle and applied to the tooth surface around exposed root dentin. When Bioglass® particles are put in contact with dentin, they adhere to the surface, rapidly form a hydroxycarbonate apatite layer and occlude the tubules, thereby relieving the pain. Studies have shown that the Bioglass® particulate performs better than current therapies in relatively low concentrations [80]. Early in 2004, FDA cleared two products for sale through the 510[k] process, and product sales began in mid-2004.

Other dental and maxillofacial applications include pulp capping [81], sinus obliteration [82] and repair of orbital floor fracture [83]. Use of 45S5 Bioglass® particulate as an injectable for treatment of urinary incontinance has also been tested *in vivo* [84].

17 Sol-gel processing

All early bioactive glass and glass ceramic processing involved melting the glass phase at high temperatures followed by casting of bulk implants or quenching of powders. In 1991, Rounan Li, Clark and Hench showed that a stable bioactive gel-glass could be made by sol-gel processing [85]. A series of compositions were studied. *In vitro* bioactivity in stimulated body fluid was demonstrated for gel-glass compositions with nearly 90% SiO_2. The rate of surface HCA formation for the 58S compositions was even more rapid than for melt derived 45S5 Bioglass® . This finding offered a potential processing method for molecular and textural tailoring of the biological behaviour of a new, third generation of bioactive materials. Particles, fibres, foams, porous scaffolds, coatings, and net shape monoliths can all be made by Sol-gel processing. Mesopores in the nanometers size range can be achieved as well as macropores in the range of 100 to 500 micrometers. Surfaces of the bioactive gel-glasses can be modified by a variety of surface chemistry methods. A long series of papers documenting these developments have been published, as documented in ref. [1]. Key review papers on sol–gel processing are refs. [86–90]. Effects of the ionic dissolution products from the bioactive gel-glasses are discussed in refs. [62, 63]. In 2000 the sol–gel processing of bioactive gel–glasses was simplified by reducing the compositions to just two components (CaO and SiO_2). Sarvanapavan and Hench showed that the $70SiO_2/30CaO$ (in mol%) system was as bioactive as 58S or 45S5 melt-derived Bioglass® [91]. This CaO-SiO_2 system is the basis for many of the third generation tissue regeneration materials presently in development [92–94].

18 Tissue engineering [TE] scaffolds

Three types of bioactive resorbable TE scaffolds are being developed for tissue engineering applications: (1) Bioactive foam scaffolds for bone tissue engineering, (2) bioactive, resorbable scaffolds for soft connective

tissue regeneration and repair, (3) hybrid inorganic/organic bioactive scaffolds. Details are given in several papers in this symposium. Also see ref. [1] for a discussion and list of references.

19 Anti-microbial bioactive gel-glasses

Bioactive glasses with silver in their composition have been developed and applications are being tested in several applications using the materials as either powders or as scaffolds [95–97]. These bioactive sol-gel derived materials release Ag ions at controlled parts per million levels. The Ag ions provide both bacteriostatic and bactericidal effects for *e-coli* and gram + and gram − bacteria without damage to human cells.

Clinical applications in wound dressings are soon to be achieved.

20 Molecular modelling of Bioglass® surface reactions

Understanding the interaction of proteins and cells with surfaces is one of the great challenges of biomaterials research. Molecular modelling of the interaction of surface sites with amino acids offers potential to understand the effectiveness of binding of charged molecules with the material surface. West and Hench used various levels of quantum mechanics based semi-empirical molecular orbital (MO) models to attack this problem. The models are based upon the knowledge gained from surface chemical analyses of the bioactive glass surface, especially the early formation of a biologically active sol-gel derived silica layer. Results from the MO calculations showed energetically favourable reaction pathways for metastable states of penta-coordinated silicon in the reaction tetrahedra that either carboxyl or amine sites on the amino acids of proteins. The findings led to a series of papers that describe an inorganic route to synthesis of polypeptide bonds. These inorganic reaction pathways might be relevant in the activation of genes or modification of cell membrane proteins that control cell cycle. See reference [98] for a review of these calculations and their relevance to understanding the behaviour of bioactive materials.

An MO model was used by Drs. Lobel, West and Hench to understand the reaction pathways for diatoms to create hydrated silica frustules using the soluble silica in sea water [99]. Similar protein template-based reactions reactions [100] might have been involved in the key genetic mutations associated with the role of soluble hydrated silica in biosilicification or onset of bone mineralization, as discussed in ref. [98]. Later *ab initio* calculations, published by Nedelec and Hench, confirmed the level of accuracy of the earlier semi-empirical models [101].

21 Technology transfer from concept to clinic

The objective of all biomaterials research is to produce improved products for clinical use. This requires transferring laboratory findings into pre–clinical animal trials and then clinical studies sufficient to pass regulatory evaluation. The first Bioglass® products took fifteen years. Some of the reasons are described in ref [1]. Briefly, the main factors are: (1) Composition of this unique glass could not be protected by patent due to early publications. (2) The tissue-implant interface could not be seen under the optical microscope. (3) Inexperience in negotiating effective technology transfer contracts. (4) Regulatory delays due to pioneering the field. (5) Learning the technology transfer process by trial and error. The experience obtained in seeing Bioglass® technology make it into clinical products was the basis for an analysis of the entire technology transfer process. This analysis was published in references [102, 103] and is summarised in the book "Sol-Gel Silica: Properties, Processing and Technology Transfer" by L. L. Hench, Noyes Publications (1998). Reference 2 expands on this process using the latest examples of successful product development.

22 Conclusion

After 30 years, as a result of satisfactory contracts and commercial relationships, Bioglass® medical and dental products are sold in 35 countries. A wide variety of other bioactive materials are also now in use world wide. Problems endangered by the barriers cited above have mostly been overcome and research using state of the art equipment is now normal. Delays, despite their cost, allowed valuable long-term data to accumulate, but it is quite clear that being a pioneer and ahead of one's time is, on the whole, not desirable.

23 Implications for the future

A cellular and molecular basis for development of third-generation biomaterials provides the scientific foundation for molecular design of scaffolds for tissue engineering and for *in situ* tissue regeneration and repair, with minimally invasive surgery. The economic advantages of these new approaches may aid in solving the problems of caring for an ageing population. It should be feasible to design a new generation of gene-activating biomaterials tailored for specific patients and disease states. Tissue-engineered constructs based on a patient's own cells may be produced that can be used to select an optimal pharmaceutical treatment. The results suggest that bioactive stimuli may be used to activate genes in a preventive treatment to maintain the health of tissues as they age. Only a few years ago this concept would have seemed unimaginable.

But we need to remember that only 35 years ago the concept of a material that would not be rejected by living tissues also seemed unimaginable. Bioglass® provides a starting point.

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