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Opening paper 2015- Some comments on Bioglass: Four Eras of Discovery and Development

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Abstract: Historically the function of biomaterials was 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; strong, stable interfacial bonding of an implant with host tissues. In the 1980's it was discovered that bioactive glasses could be used in particulae form to stimulate osteogenesis which thereby led to the concept of regeneration of tissues. This article summarizes the four eras of development of bioactive glasses that have led from concept of bioactivity to widespread clinical and commercial use, with emphasis on the first composition, 45S5 Bioglass®. The four eras are; A) Era of Discovery, B) Era of Clinical Application, C) Era of Tissue Regeneration, and D) Era of Innovation. Key scientific and technological questions answered for the first three eras are presented. Questions still to be answered for the fourth era are included to stimulate innovation in the field.

1 Introduction

It is an honor to present this opening paper in the inaugural edition of the journal *Biomedical Glasses*. This new Journal is timely. Numerous clinical applications in diverse fields routinely use bioactive glasses. Results are reported in a wide variety of journals. Having a journal devoted exclusively to the field will make it much easier to follow clinical performance and compare clinical findings. Likewise, many new concepts in the design and processing of inorganic glass-based bioactive materials, such as inorganic-organic hybrids, are being developed leading to the possibility of matching bio-mechanical properties of tissues

and load bearing applications with biological properties. This journal will provide an excellent source of literature to follow development of these new bioactive materials. This new journal will also provide a standard source of literature to focus on new concepts of use of bioactive materials for tissue regeneration, as discussed below.

As the field moves forward we much remember that the purpose of all early, first generation, biomaterials was to *replace* diseased, damaged or ageing tissues. The materials were selected to match as closely as possible the physical properties of the replaced tissues with minimal toxic response in the host; ie. be as “bioinert” as possible. More than 50 types of implants made from 40 different first generation biomaterials are used annually to improve the quality of life of millions of people worldwide. This success clinically provides a standard for comparison of all new biomaterials, including medical glasses. However, large numbers of patients are now outliving prostheses made of first generation biomaterials.

A new approach to tissue repair or replacement is now possible through the concept of *tissue regeneration*. This concept of regeneration of tissues instead of replacing them is possible in part to the development of second and third generation bioactive materials, especially bioactive glasses. In 1969 began the development of second generation bioactive materials capable of bonding to hard and soft connective tissues [1–13] and third generation bioactive resorbable materials, specifically designed for tissue regeneration [14–19].

The objective of this paper is to review briefly the questions answered in three eras of development of bioactive glasses from the discovery in 1969 to the present, 2014. The three eras are; A) Era of Discovery, B) Era of Clinical Application, C) Era of Tissue Regeneration. Several important unanswered questions for the fourth era, D) Era of Innovation will also be suggested. Answers should appear in the journal in the years ahead.

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Table 1: Composition and Properties of Bioactive Glasses and Glass-Ceramics Used Clinically for Medical and Dental Applications

Composition (wt.%)	45S5 Bioglass (NovaBone, Perioglas) (NovaMin)	S53P4 (AbminDent1) (BonAlive)	A-W Glass-ceramic (Cerabone)
Na ₂ O	24.5	23.0	0
CaO	24.5	20.0	44.7
CaF ₂	0	0	0.5
MgO	0	0	4.6
P ₂ O ₅	6.0	4.0	16.2
SiO ₂	45.0	53.0	34.0
Phases	Glass	Glass	Apatite Beta-wollastonite Glass
Class of Bioactivity	A*	A	B

2 Era of Discovery (1969-79)

A characteristic of a “bioinert” tissue response is formation of a non-adherent fibrous capsule at the materials-host interface. In 1969 Hench, Splinter, Allen and Greenlee discovered that certain compositions of Na₂O-CaO-P₂O₅-SiO₂ glasses formed a strong, adherent bond to bone [1]. These biomaterials have become known as “bioactive”, with a controlled reaction in the physiological environment that leads to bonding of living tissues to a non-living man-made implant [1–13]. The discovery of bioactive bonding was the result of a research proposal submitted to the US Army Medical R and D Command in 1968 by Drs. Hench, Allen and Greenlee 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.*”

The proposal was funded for a one year test of the hypothesis. Dr. Hench designed the first glass compositions (Table 1) for testing in a rat femoral implant model designed by Dr. Ted Greenlee at the University of Florida, Department of Orthopaedics [1–3].

The implants were made in the Department of Materials Science and Engineering and inserted into 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 experimental 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 with an osteotome and they do not move. Control implants easily slip out of their fibrous capsule but the special ceramic implants are firmly bonded to bone.”

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 [1–3]. The *in vitro* tests showed that the 45S5 Bioglass® composition (see Table 1) developed a hydroxyapatite (HA) layer in test solutions. This HA phase developed on the surface of the implants *in vitro* was equivalent to the interfacial HA crystals observed *in vivo* by Dr Greenlee’s transmission electron micrographs of the bonded interface. The HA crystals *in vivo* 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 [1–13].

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 occurs between living tissues and non-living implant materials [11]. These questions are listed below with very brief summary answers. Most of 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, as summarized by Hench, Wilson and Greenspan in their comprehensive review, “Bioglass: A Short History and Bibliography” [11].

A key review article that summarizes the answers to most of the questions listed above was published in 1982. It is reference 9; “Adhesion to Bone” by L.L. Hench and A.E. Clark, in *Biocompatibility of Orthopaedic Implants*, D.F. Williams and G.D. Winter, eds, CRC Press. The paper documents in Part A the time sequence of Bonding of Bioglass® in Rat Femur and Tibia, based upon citations reviewed in reference 11. In Part B, Bonding of Bioglass® Implants to the Femur in Canine and Monkey Bones is summarized [9, 11]. Part C reviews the data of Bonding of Mandibular and Maxillar Bone of Primates and Swine to Bioglass® implants [9, 11]. A stable bone bonded implant in the anterior region of the mandible of a baboon after four years of functional use is presented in this paper, one of the longest *in vivo* studies of biomaterials in primates ever published [9].

There are two important aspects of the questions explored in the Era of Discovery. First the methodology for investigating the reactive glass surface and bonded interfaces of bioactive implants with living tissues had to be developed as there was no precedence for such analyses. Thus, instrumental techniques such as infrared reflection spectroscopy, developed by Sanders and Hench, and applied to bioactive glasses was a critical part of this early effort as was cryogenic Auger electron spectroscopy (AES), developed by Ouichi, Pantano, Ogino and Hench [20–23]. The extensive bibliography in the Hench, Wilson and Greenspan review provides the many citations that document the methods used to answer the questions listed below [11].

The second aspect of this early era was an emphasis on questions related to use of bioactive glass or glass-ceramics as replacement body parts. Thus, tests were conducted primarily on bulk samples or as bioactive coatings on high strength metal, 316L or Co-Cr alloys, or ceramic, alumina, implants [11–13]. The questions assumed that the eventual applications of bioactive bonding would be to replace a diseased, damaged or missing part of the body. The second Era of Clinical Applications was based upon this knowledge.

3 Era of Discovery Questions

Answered:

(1) What is the physical, chemical and biological nature of the bioactive bond?

The interfacial bond between a bioactive glass and bone is composed of hydroxycarbonate apatite (HCA) crystals bonded to collagen fibers [1–3].

(2) What are the mechanisms involved in formation of the bioactive bond?

A sequence of five surface reactions occur on the glass surface; [11–13] a) ion exchange of sodium ions with protons and hydronium ions, b) silanols formed from the ion exchange undergo a condensation reaction to form a high surface area silica gel, c) calcium and phosphate ions from body fluids along with ions released from the glass form an amorphous calcium phosphate phase on top of the silica gel, d) crystallization of the calcium phosphate rich phase occurs incorporating carbonate ions from solution to form poly crystalline HCA. The HCA crystals bind to collagen from the host tissue to form the bond.

(3) How rapidly does the bioactive bond form?

The rate of bonding depends upon species of animal and location of implant. Bonding to rat femora occurs in days, bonding to primates, including humans occurs within weeks [9].

(4) How thick is the bioactive bond?

The bond to bone is >100 micrometers within a few weeks and stabilizes at approximately 200-300 micrometers by approximately six months [24].

(5) What is the mechanical strength of the bioactive bond?

The strength of the bond is equal or stronger than host bone [6, 9].

(6) How mechanically rigid or compliant is the bioactive bond?

Due to microporosity of the thick silica gel and calcium phosphate bi-layer on the glass surface the interfacial bond has a gradient of elastic modulus from the glass to the tissue that mimics the gradient of elastic compliance between tendons and ligaments and bone [24].

(7) How stable is the bioactive bond when exposed to aging or disease processes?

Limited experiments show that the bond when formed remains stable for many years [9, 11, 24]. Clinical findings, see below, show stability for >10 years without interfacial failures to either bone or soft tissue bonded interfaces [25].

(8) Will the bioactive bond form in the presence of mechanical motion?

The interface must remain immobile for the bond to form, thus devices require a close mechanical fit in the host bone or soft tissues [10].

(9) Will the bioactive bond form in the presence of infection?

The bioactive glass ion exchange process increases the interfacial pH to alkaline levels and renders the material bactericidal making it ideal to use in sites where pathogens are present [10, 11].

(10) Is the rate of bioactive bond formation, properties of the bond or bond stability influenced by composition of the implant material?

The grandfather 45S5 Bioglass composition, with 45 weight % SiO_2 , lies at the center of a bioactivity compositional diagram composed of SiO_2 -CaO- Na_2O with a constant 6% P_2O_5 , all in weight %, Table 1. The upper boundary for Class A bioactivity where bonding to both bone and soft tissue is possible is at 52% SiO_2 . Compositions between 52 and 60% SiO_2 exhibit slower rates of bonding and bond only to bone, Class B bioactivity. The upper boundary of bioactivity and bone bonding is at 60% SiO_2 (Fig. 1)[11–13, 24].

(11) Can bioactive bonding be obtained at the interface with prostheses that will withstand functional loads?

Bonding of 45S5 Bioglass implants to monkey femora in a segmental bone replacement model and to femoral stem prostheses with normal weight bearing loading of the implants has been shown to be stable. Mechanical tests of the

implants show interfacial strength to be equal or greater than host bone [6–9].

(12) What is the nature of other tissue reactions when the bioactive bonding was primarily formed in contact with bone?

45S5 Bioglass implants in soft tissues will form a stable bond if the interface is immobile for sufficient time for collagen to become incorporated with the growing HCA layer on the glass [10]. Thickness of the soft tissue-bioactive glass bond is greater than bone interfacial bonds and the rate of formation of the soft tissue interfacial bond is more rapid. The soft tissue bond compositional limit for bioactivity is the same as for Class A bioactivity [24–30].

(13) When and where was bioactive bone bonding confirmed?

Confirmation of bonding of Bioglass to bone was achieved in 1976 by Peter Griss, Professor of Orthopedics, in Heidelberg, Germany. Bioglass-coated alumina implants developed by Greenspan and Hench at the University of Florida were tested as load bearing femoral stem and acetabular cup hip prostheses in sheep. The results showed bone bonding of femoral stems and cups with the Bioglass coated implants but the coatings were not stable [31].

Additional confirmation of bonding of bioactive glasses to bone and their clinical effectiveness was led by Andersson, Karlsson, Yli-Urpo and colleagues at Abo Academy and University of Turku, Finland [32]. A comprehensive series of glasses was designed in the 1980s and implanted in animal models. Glasses within similar compositional range to 45S5 Bioglass (Table 1), called S53P4 (BonAlive) also bonded to bone with long term stability. Glasses outside the bioactive boundary did not bond. Clinical use of the Finnish-derived bioactive glass S53P4 in head, neck and spinal surgical repair has been successful for many years [32–35, 35].

(14) Can bioactive implants be made strong and tough?

An important modification of bioactive glasses was the development in the 1980s of A/W (apatite/wollastonite) bioactive glass-ceramic by Professors T. Yamamuro and T. Kokubo and colleagues at Kyoto University, Kyoto, Japan [24, 37, 38]. A unique processing method produced a

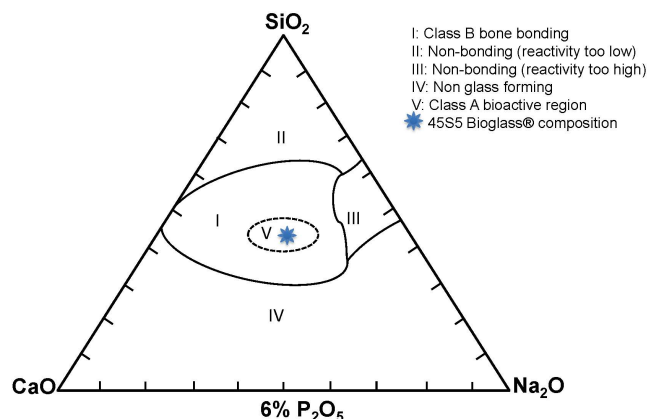


Figure 1: Compositional dependence of bone and soft tissue bonding to bioactive glasses. Dr. Wilson continued investigation of the interfacial interaction of soft tissues established, in key papers with David Nolletti, the compositional dependence of the bonding of bioactive glasses to soft tissues.

very fine-grained glass-ceramic composed of very small apatite and wollastonite crystals bonded by a bioactive glass interface. Mechanical strength, toughness and stability of A/W glass-ceramics in physiological environments are excellent. Bone bonded to A/W implants with high interfacial bond strengths. Animal tests led to approval to use the A/W material in numerous orthopaedic applications in Japan with high levels of success, especially in vertebral replacement [37, 38].

4 Era of Clinical Application (1980-95)

A key paper by Wilson *et al.* “Toxicology and Biocompatibility of Bioglass®”, in the Journal of Biomedical Materials Research, 15, 6, 805-817 (1981) established that soft connective tissues could also form a bond to 45S5 Bioglass [10]. This is one of the most important papers in the history of Bioglass for two reasons. The discovery of soft tissue bonding is one. Evidence of rapid, stable and strong bonding of collagen from soft tissues paved the way for development of the first bioactive glass clinical applications that required both stable bone and soft tissue interfaces. These devices are considered second generation devices made of bioactive glass with the objective of replacing diseased, damaged or missing body parts.

A second important feature of the Wilson *et al.* paper is extensive documentation of results of sixteen *in vitro* and *in vivo* tests that established the safety of use of particulate forms of Bioglass as well as bulk implants [10]. 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, as well as application for regulatory approval of commercial sales of these devices by the FDA and a CE mark from the EU [12].

5 Era of Clinical Application Questions Answered

(15) Are devices made from bioactive glass safe?

The Wilson *et al.* paper cited above provided evidence that various compositions of $\text{Na}_2\text{O}-\text{CaO}-\text{P}_2\text{O}_5-\text{SiO}_2$ glasses within the Class A bioactivity range shown in Figure 1 do not exhibit toxicological responses regardless of form of the glass [10, 11]. Particles, fibers and bulk samples with a wide range of dimensions exposed to a wide range of cell culture studies were all non-toxic, in some cases superior to control materials [10].

(16) Are implants made from bioactive glass biocompatible?

The Wilson *et al.* paper cited above also showed that various compositions of $\text{Na}_2\text{O}-\text{CaO}-\text{P}_2\text{O}_5-\text{SiO}_2$ glasses within the Class A bioactivity range shown in Figure 1 when used as implants in various forms of the glass functioned in a stable manner in a variety of animal models. There was equivalent behaviour of a stable bonded bone and soft tissue interface regardless of species, including mice, rats, rabbits, dogs, sheep, pigs, monkeys and baboons [9–12, 26].

(17) What were the first clinical products made of Bioglass?

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 [12, 27–30]. The device was called the “Bioglass® Ossicular Reconstruction Prosthesis”, and tradenamed ‘MEP®’. The device was cleared by the U.S. Food and Drugs Administration (FDA) 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 tis-

sue (tympanic membrane) as well as bone tissue [28]. Other uses in head and neck surgery of bioactive glasses are described in reference 11.

The second Bioglass device to be placed into the market was the Endosseous Ridge Maintenance Implant (ERMI[®]), which was cleared by the FDA via the 510(k) process, in November, 1988 [12]. The device was intended 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 [40–45]. 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.

(18) How successful were the first Bioglass clinical products?

In several reviews of clinical studies, it was shown that the Bioglass MEP[®] outperformed other bioceramic and metal prostheses [31]. Most other types of middle ear prostheses were lost by extrusion after a few years. In contrast, Bioglass middle ear devices formed a stable bond to both bone, such as the stapes footplate and the soft tissues of the tympanic membrane and thus remained stable for more than 10 years as reported in follow-up studies at both the University of Florida and Guy's Hospital in London [12, 31, 46, 47].

Equivalent long term, >10 years, success of the Bioglass ERMI's were reported by Stanley et. al. Alternative Class B bioactive implants made of synthetic HA were lost by extrusion or exfoliation from the jaw after only a few years post implantation. In contrast, 45S5 Bioglass implants maintained stable bonding in alveolar bone and a stable gingival interface for long term and maintained thickness of the bone without resorption generally experienced by denture wearers [40–44].

6 Era of Tissue Regeneration (1985-2005)

(19) What experiment led to the discovery of osteoproduction (osteostimulation) and the concept of using Bioglass particulate for regeneration of bone?

The first paper to describe potential use of 45S5 Bioglass[®] particulate in repair of bone was published in 1987 by Wil-

son, Low, Fetner and Hench, Department of Periodontology and Bioglass Research Center, University of Florida [48]. The paper described the effect of various sizes of Bioglass particulate on regeneration of bone in periodontal defects created in a monkey model. The seminal finding was the stimulation of new bone throughout the defect. Bone growth was initiated at the surface of the bioactive glass particles and rapidly formed connections between the particles regenerating a trabecular bone network that mimicked the original trabecular bone of the jaw prior to creating the defect. The study showed that there was an optimal rate of bone repair when a range of particle sizes of Bioglass was used. The results also showed that bone regeneration was sufficiently rapid that it prevented encapsulation of the site by epithelial tissues. This paper and follow-up publications by Wilson and Low [49] provided the foundation for a clinical trial in patients at the University of Florida that led to FDA regulatory approval of the use of bioactive glass particulate for periodontal repair [12].

(20) How was it possible to quantify and compare the rate of bone regeneration for different bioactive materials?

Quantification and comparison of the effect of bioactive glass on regeneration of bone was based upon a series of important studies conducted by Dr. Oonishi et al. in Osaka, Japan [50, 51]. The Oonishi investigations used a critical size defect in a rabbit femoral condyle model to compare rates of bone formation in the presence of different types of bioceramics particles of the same particle size.

Details of evidence to support the distinction of Class A and Class B bioactive materials along with a description of the temporal sequence of material and cellular events involved in the regeneration of bone by Class A bioactive materials are in numerous reviews [12–14, 52, 53]. Many studies have led to the conclusion that there are twelve reaction stages involved in the regeneration of bone at the interface of a Class A bioactive glass. The sequence and time required for these twelve reaction steps are summarized in Figure 2.

(21) In the Oonishi critical size defect model in rabbits how rapidly did 45S5 Bioglass regenerate bone?

The studies showed there is more bone formed in just one week in the presence of bioactive glass 45S5 than is formed when synthetic hydroxyapatite (HA) or other calcium-phosphate ceramic particulates are placed in the

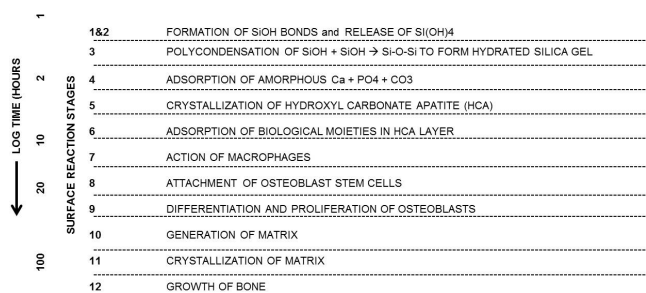


Figure 2: Twelve stages of reaction to form new bone (osteogenesis) bonded to a 45S5 bioactive glass surface. Stages 1-5 are controlled by the kinetics of surface reactions. Note the logarithmic time axis on the left. The early stages of glass surface reactions (Stages 1-4) occur within minutes to a few hours on the most bioactive glass surfaces with compositions ranging from 45 to 52% silica.

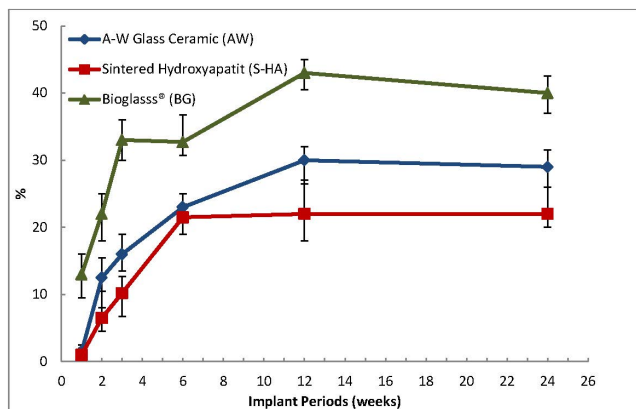


Figure 3: Rapid regeneration of new bone from Class A Bioglass versus Class B bioactive A/W and HA particle particles in Oonishi critical size defect rabbit condyle model. (Adapted from [51]). Note rapid growth of new bone within 4 weeks in the presence of Bioglass particles and eventual greater amount of bone regenerated within the critical size defect.

same type of defect for several weeks, Figure 3 [50, 51]. After several weeks of bone regeneration there is almost twice as much new bone present in the defect containing bioactive glass. By 12 weeks the amount of bone that is regenerated by Bioglass particles matches that originally present in the site. The architecture of the trabecular bone is also equivalent to the original bone. In other studies Wheeler and colleagues demonstrated that the mechanical properties of the defect site have been restored by the regenerated bone [53], as discussed in reviews [12, 52, 53]. These large differences in rates of *in vivo* bone regeneration and extent of bone repair confirm that there are two classes of bioactive materials, Class A and Class B [12, 52].

(22) What is the difference between Class A and Class B bioactive materials?

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. Class B bioactive materials bond to bone via osteoconduction but do not bond to soft tissues as the surface reactions to form a HCA layer are too slow to bind collagen fibers of soft tissues [12–14, 52, 53].

Class A bioactivity leads to both osteoconduction and osteostimulation of new bone; i.e., enhanced osteogenesis as a consequence of rapid reactions on the bioactive glass surface [12–14, 52, 53]. 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 A bioactive materials bond to both bone and soft connective tissues as the surface reactions to form a HCA layer are very rapid and the HCA layer forms quickly to bind collagen fibers of soft and hard tissues.

(23) What is the effect of Bioglass on cell cycle of primary bone cell cultures?

Seminal papers by Xynos *et. al.* established that there is control of the cycle of a mixed population of cells as well as genetic control of the cellular response when the cells are exposed to the surface of bioactive glasses (45S5 Bioglass) or the ionic dissolution products released from the glass surface [15–17]. Cells that are not capable of differentiation into a mature osteoblast phenotype are switched into apoptosis by the ionic stimuli or bulk Bioglass surfaces eliminating them from the culture environment within the first days of exposure to the bioactive stimuli.

(24) What genes are activated or up-regulated in osteoblast progenitor or stem cells exposed to ionic stimulation products released from 45S5 Bioglass?

Seven families of genes are up-regulated when primary human osteoblasts are exposed to the ionic dissolution products of bioactive glasses [18–20]. The gene expression occurs within 48 hours, and includes enhanced expression by more than 2-fold of the families of genes listed in Table 2.

Table 2: Families of genes up-regulated or activated by ionic dissolution products from bioactive glass

1) Transcription factors and cell cycle regulators	2 to 3.7 fold
2) Signal transduction molecules	2 to 7 fold
3) Proteins in DNA synthesis, repair, recombination	2 to 3.2 fold
4) Growth factors and cytokines	2 to 3 fold
6) Extracellular matrix components	2 to 6 fold
7) Apoptosis regulators	2 to 5 fold
5) Cell surface antigens and receptors	1.6 to 4.5 fold

(25) What is the function of the genes activated or up-regulated in the presence of bioactive ionic dissolution products from Bioglass?

The up-regulated genes encode nuclear transcription factors and cell cycle regulators [18–20]. Potent growth factors, especially insulin-like growth factor II (IGF-II), were increased by 3.2 fold along with IGF binding proteins and proteases that cleave IGF-II from their binding proteins. Similar bioactive induction of the transcription of at least five extracellular matrix components (2 to 3.7 fold) and their secretion and self-organization into a mineralized matrix is responsible for the rapid formation and growth of bone nodules and differentiation of the mature osteocyte phenotype [18–20].

(26) How general are the findings of effect of bioactive stimuli on gene expression of osteoprogenitor and stem cells?

Subsequent studies confirmed the results of the early Xynos et.al. findings and extended the generality to include several types of precursor cells and differing sources of biologically active Ca and Si ionic stimuli [55–57]. Bone biology and gene array analyses of five different in-vitro models using four different sources of inorganic ions provide the experimental evidence for a genetic theory of osteogenic stimulation. All experiments showed enhanced proliferation and differentiation of osteoblasts towards a mature, mineralizing phenotype without the presence of any added bone growth proteins, such as bone morphogenic proteins (BMPs). Shifts in osteoblast cell cycles were observed as early as six hours for most experiments, with elimination (by apoptosis) of cells incapable of differentiation. The remaining cells exhibited enhanced synthesis and mitosis. The cells quickly committed to generation of extracellular matrix (ECM) proteins and mineralization of the matrix. The expansion of the research field is leading to the development of alternative bioactive glass formulations incorporating other biologically active

ions [58], and there is emerging in vivo and in vitro evidence on the effects of bioactive glass dissolution products on angiogenesis [59].

7 Era of Innovation (2000-2020)

There are many challenges still ahead for the field of medical glasses that require advances in a fourth era; an era of innovation. Significant scientific and technological issues remain unanswered, such as:

- 1) Tissue engineered constructs for replacement of large bone defects have been investigated since the beginning of this era around year 2000 but are still not available as routine clinical products. Is it possible to achieve a stable vasculature *in situ* in tissue engineering constructs that can be maintained in culture before implantation or be generated *in vivo* following implantation?
- 2) Load bearing devices that can be used in orthopedics with long term, predictable reliability and bond to living bone without stress shielding are still not available clinically. Is it feasible to produce and test bioactive implants that have predictable 20 year lifetime survivability under simulated load bearing physiological conditions?
- 3) Numerous soft tissue engineering applications have been investigated at an exploratory level but still require development into clinical products. Is it possible to obtain regulatory approval for clinical trials of soft tissue applications based upon limited *in vitro* and *in vivo* data and lack of understanding of basic biological mechanisms of soft tissue response to bioactive materials?
- 4) Control of stem cell technology to use with tissue engineering scaffolds is in its infancy. What are the fundamental mechanisms of stimulation of stem cell differentiation towards specific phenotypes and can these mechanisms be controlled to achieve

greater than 99.999% accuracy to avoid potential tumorigenesis?

- 5) Design and production of bioactive materials with tailored bio-mechanical properties that are bioactive and rapidly incorporated with living tissue are exciting possibilities but can they be developed into clinical devices with predictable long term performance?
- 6) As discussed above, tissue regeneration via gene activation is a clinical reality that leads to enhanced osteogenesis but what are the fundamental mechanisms involved at the nucleus in the cell?

8 Conclusion

Until the questions related to the topics above, and more, are answered, applying the concept of bioactive ionic stimulation broadly to a wide range of regenerative medicine is largely trial and error. A general theory of bioactivity at the gene expression level still waits. The long term potential for new clinical applications for medical glasses is extraordinary. Achieving this potential is a great challenge with enormous socio-economic pay-off ahead. The need is great. The concepts and stimuli exist. The incentives are real. Will the field rise to meet this challenge?

A challenge for authors of this journal is to avoid pursuing small incremental advancements in this exciting field. Instead, authors should strive for unique and innovative approaches at a fundamental molecular biology level to create new bioactive materials and test them in representative biological conditions that mimic their use clinically. The goal must be to create materials that are revolutionary and can improve the quality of life and care for our ageing population without increasing the cost of care. This is a goal worth striving for and a vision that will last for decades.

References

- [1] L.L. Hench, R.J. Splinter, W.C. Allen, and T.K. Greenlee, Jr. Bonding Mechanisms at the Interface of Ceramic Prosthetic Materials, *J. Biomed. Mater. Res.* 2[1] (1971), 117-141.
- [2] C.A. Beckham, T.K. Greenlee Jr. and A.R. Crebo, Jr Bone Formation at a Ceramic Implant Interface, *Calc.Tissue Res.* 8, 165 (1971)
- [3] T.K. Greenlee Jr., C.A. Beckham, A.R. Crebo and J.C. Malmberg, Glass Ceramic Bone Implants, *J. Biomed. Mater. Res.* 6 (1972), 235-244

- [4] L.L. Hench and H.A. Paschall., Direct Chemical Bonding of Bioactive Glass-Ceramic Materials and Bone, *J. Biomed. Mater. Res. Symp. No. 4* (1973), 25-42.
- [5] L.L. Hench and H.A. Paschall , Histo-Chemical Responses at a Biomaterials Interface, *J. Biomed. Mater. Res. No. 5 (Part 1)* (1974), 49-64.
- [6] G. Piotrowski, L.L. Hench, W.C. Allen and G.J. Miller, Mechanical Studies of the Bone-Bioglass Interfacial Bond, *J. Biomed. Mater. Res. Symp. 9[4]* (1975), 47-61.
- [7] L.L. Hench, H.A. Paschall, W.C. Allen and G. Piotrowski, Interfacial Behavior of Ceramics Implants, National Bureau of Standards Special Publication 415, May 1975, 19-35.
- [8] A.E. Clark, L.L. Hench and H.A. Paschall, The Influence of Surface Chemistry on Implant Interface Histology: A Theoretical Basis for Implant Materials Selection, *J. Biomed. Mater. Res.* 10 (1976), 161-174.
- [9] L.L. Hench and A.E. Clark, Adhesion to Bone, in *Biocompatibility of Orthopaedic Implants*, D.F. Williams and G.D. Winter, eds., CRC Press. Boca Raton, Florida, Vol. II, 1982, Chapter 6.
- [10] J. Wilson, G.H. Pigott, F.J. Schoen, and L.L. Hench, Toxicology and Biocompatibility of Bioglass, *J. Biomed. Mater. Res.* 15, 805 (1981).
- [11] L.L. Hench, The story of Bioglass, *J. Mater. Sci: Mater Med.* 17 (2006), 967-978.
- [12] L.L. Hench, June W. Hench and D.C. Greenspan, Bioglass: A Short History and Bibliography, *J. Aust. Ceram. Soc.* 40[1] (2004), 1-42.
- [13] L.L. Hench, Bioceramics: From Concept to Clinic, *J. Am. Ceram. Soc.* 74[7] (1991), 1487-1510.
- [14] L.L. Hench, Bioceramics., *J. Am. Ceram. Soc.* 81[7] (1998), 1705-28.
- [15] I.D. Xynos, M.V.J. Hukkanen, J.J. Batten, I.D. Buttery, L.L. Hench and J.M. Polak, Bioglass® 45S5 Stimulates Osteoblast Turnover and Enhances Bone Formation In Vitro: Implications and Applications for Bone Tissue Engineering., *Calcif. Tiss. Int.* 67: 321-329 (2000)
- [16] L.L. Hench, J.M. Polak, I.D. Xynos and L.D.K. Buttery, Bioactive Materials to Control Cell Cycle, *Mat. Res. Innovat.* 3:313-323 (2000).
- [17] I.D. Xynos, A.J. Edgar, L.D. Buttery, L.L. Hench and J.M. Polak, Ionic Dissolution Products of Bioactive Glass Increase Proliferation of Human Osteoblasts and Induce Insulin-like Growth Factor II mRNA Expression and Protein Synthesis, *Biochem. Biophys. Res. Comm.* 276: 461-465 (2000).
- [18] I.D. Xynos, A.J. Edgar, L.D.K. Buttery, L.L. Hench and J.M. Polak, Gene Expression Profiling of Human Osteoblasts Following Treatment with the Ionic Dissolution Products of Bioglass® 45S5 Dissolution, *J. Biomed. Mater. Res.* 55: 151-157 (2001).
- [19] L.L. Hench, I.D. Xynos, A.J. Edgar, L.D.K. Buttery, J.M. Polak, J.P. Zhong, X.Y. Liu and J. Chang, Gene Activating Glasses, *Journal of Inorganic Materials* 17 (2002), 897-909.
- [20] L.L. Hench, Glass and Genes: The 2001 W.E.S. Turner Memorial Lecture, *Glass Technology* 44 (2003), 1-10.
- [21] L.L. Hench, Characterization of Glass Corrosion and Durability, *J. Non-Crystalline Solids* 19 (1975) 27-39.
- [22] A.E. Clark Jr., C.G. Pantano Jr. and L.L. Hench, Auger Spectroscopic Analysis of Bioglass Corrosion Films, *J. Am. Ceram. Soc.* 59[1-2] (1976), 37-39.
- [23] Cheol Y. Kim, A.E. Clark and L.L. Hench, Early Stages of Calcium-Phosphate Layer Formation in Bioglass, *J. Non-Crystalline*

- Solids 113 (1989), 195-202.
- [24] L.L. Hench and D.E. Clark, Physical Chemistry of Glass Surfaces, *J. Non-Crystalline Solids* 28[1] (1978), 83-105.
- [25] L.L. Hench, ed, An Introduction to Bioceramics-Second Edition, chapters 6,7,8,9,10,11,12, 31, Imperial College Press, London, (2013)
- [26] H.R. Stanley, L.L. Hench, C.G. Bennett Jr., S.J. Chellemi, C.J. King III, R.E. Going, N.J. Ingersoll, E.C. Ethridge, K.L. Kreutziger, L. Loeb and A.E. Clark, The Implantation of Natural Tooth Form Bioglass® in Baboons - Long Term Results, *Intern. J. Oral Implantology*, 2 (1981), 26-36.
- [27] Gerald E. Merwin, James S. Atkins, June Wilson, Larry L. Hench, Comparison of Ossicular Replacement Materials in a Mouse Ear Model, *Otolaryngol. Head Neck Surg.* 90 (1982) 461-469.
- [28] June Wilson, J.S. Atkins, G.E. Merwin and L.L. Hench, Histopathological Evaluation of Interaction between Tympanic Membrane and Implant Materials, *Trans. Soc. For Biomat.* Vol.8 195, 1985
- [29] G. Merwin, L. Rogers, June Wilson and R. Martin, Facial Bone Augmentation using Bioglass® in Dogs., *Arch. Otolaryngology, Head and Neck Surgery.*, Vol. 112, 280-285, 198
- [30] G.E. Merwin, Bioglass® middle ear prosthesis: preliminary report, *Ann. Otol. Rhinol. Laryngol.* 95; 1 Pt 1:78-82 January-February (1986).
- [31] K. Lobel, Ossicular Replacement Prostheses, in *Clinical Performance of Skeletal Prostheses*, Hench and Wilson (eds), Chapman and Hall, Ltd, London, (1996), pp. 214-236
- [32] P. Griss, D.C. Greenspan, G. Heimke, B. Krempien, R. Buchinger, L.L. Hench and G. Jentschura, Evaluation of a Bioglass Coated Al₂O₃ Total Hip Prosthesis in Sheep, *J. Biomed. Mater. Res.* 10[4] (1976), 511-518.
- [33] O.H. Andersson, G. Liu, K. Kangasniemi and J. Juhanjoa, Evaluation of the Acceptance of Glass in Bone, 1. *Mater. Sci., Mater. in Med.* 3 (1992), 145-150.
- [34] N.C. Lindfors, J.T. Heikkila, I. Koski, K. Mattila and A.J. Aho, Bioactive glass and autogenous bone as bone graft substitutes in benign bone tumors., *J. Biomed. Mater. Res. B. Appl. Biomater.*, 90 (2009), 131-6.
- [35] J. McAndrew, C. Efrimescu, E. Sheehan and D. Niall, Through the looking glass; bioactive glass S53P4 (BonAlive®) in the treatment of chronic osteomyelitis., *Ir. J. Med. Sci.*, 182 (2013), 509-11.
- [36] K. Perna, I. Koski, K. Mattila, E. Gullichsen, Heikkila J, Aho A, Lindfors N. Bioactive glass S53P4 and autograft bone in treatment of depressed tibial plateau fractures - a prospective randomized 11-year follow-up, *J Long Term Eff Med Implants* 21 (2011), 139-48.
- [37] J. Sarin, R. Grenman, K. Aitasalo and J. Pulkkinen, Bioactive glass S53P4 in mastoid obliteration surgery for chronic otitis media and cerebrospinal fluid leakage, *Ann. Otol. Rhinol. Laryngol.* 121 (2012), 563-9.
- [38] T. Kokubo, S. Ito, S. Sakka and T. Yamamuro, Formation of a High-Strength Bioactive Glass - Ceramic in the System MgO-CaO-SiO₂-P₂O₅, *J. Mater. Sci.* 21, 536 (1986).
- [39] T. Yamamuro, A/W Glass-Ceramics: Clinical Applications, Introduction to Bioceramics, 2nd Edition, L.L. Hench, ed, Imperial College Press, Chapter 14, 189-208 (2013).
- [40] H.R. Stanley, Alveolar Ridge maintenance using endosseously placed Bioglass (45S5) Cones, *Encyclopedic, Handbook of Biomaterials and Bioengineering; Part B: Applications Volume 2*, D.L. Wise et al. (eds), (1995) Marcel Dekker Inc, New York, NY, pp. 1559-1616
- [41] H.R. Stanley, A.E. Clark and L.L. Hench, Alveolar Ridge Maintenance Implants, *Clinical Performance of Skeletal Prostheses*, L.L. Hench and June Wilson, Eds. Chapman and Hall, Publishers, London, England, (1995) 255-270
- [42] M.B. Hall, H.R. Stanley, C. King, F. Colaizzi, D. Spilman and L.L. Hench, Early Clinical Trials of 45S5 Bioglass® for Endosseous Ridge Maintenance with a New Endosseous Implant Material, *J. Prosthetic Dentistry*, 58[5] (1987), 607-613
- [43] Harold R. Stanley, Matthew B. Hall, Arthur E. Clark, Caleb J. King III and Larry L. Hench, Joseph J. Berte, Using 45S5 Bioglass® Cones as Endosseous Ridge Maintenance Implants to Prevent Alveolar Ridge Resorption: A 5-Year Evaluation, *Int. J. Oral Maxillofac. Implants*, 12 (1997), 95-105.
- [44] June Wilson, A.E. Clark, M. Hall and L.L. Hench, Tissue Response to Bioglass® Endosseous Ridge Maintenance Implants, *J. Oral Implantology*, XIX[4], (1993), 295-302
- [45] A.M. Weinstein, J.J. Klawitter and S.D. Cook, Implant-bone interface characteristics of bioglass dental implants, *J. Biomed. Mater. Res.*, 14, 1:23-9, January (1980).
- [46] J. Wilson, E. Douek and K. Rust, Bioglass® Middle Ear Devices: Ten year Clinical Results, *Bioceramics* 8, (1995) J. Wilson, L.L. Hench and D. Greenspan, Eds. Elsevier Science, Oxford, England
- [47] K.R. Rust, G.T. Singleton, J. Wilson, P.J. Antonelli, Bioglass middle ear prosthesis: long-term results, *Am. J. Otol.*, 17 (1996), 371-4.
- [48] J. Wilson, S. Low, A. Fetner and L.L. Hench, Bioactive Materials for Periodontal Treatment: A Comparative Study, in *Biomaterials and Clinical Applications*, A. Pizzoferrato, P.G. Marchetti, A. Ravaglioli and A.J.C. Lee, eds., Elsevier Science Publishers, Amsterdam, (1987), 223-228.
- [49] J. Wilson and S.B. Low, Bioactive Ceramics for Periodontal Treatment: Comparative Studies in the Patas Monkey, *J. Appl. Biomaterials*, Vol 3 (1992), 123-169
- [50] H. Oonishi, L.L. Hench, J. Wilson, F. Sugihara, E. Tsuji, S. Kushitani and H. Iwaki, Comparative Bone Growth Behaviour in Granules of Bioceramic Materials of Various Sizes, *J. Biomed. Mater. Res.* 44 (1999), 31-43
- [51] H. Oonishi, L.L. Hench, J. Wilson, F. Sugihara, E. Tsuji, M. Matsuwura, S. Kin, T. Yamamoto and S. Mizokawa, Quantitative Comparison of Bone Growth Behaviour in Granules in Bioglass®, A-W Glass Ceramic, and Hydroxyapatite, *J. Biomed. Mater. Res.* 51, (2000), 37-46.
- [52] J.R. Jones, Review of bioactive glass: From Hench to Hybrids, *Acta Biomater*, 9[1] (2012), 4457-4486.
- [53] L.L. Hench, ed, Introduction to Bioceramics, 2nd edition, Imperial College Press, (2013) Chapters 6,7,8,9,10,11,12,31
- [54] D. Wheeler, E.J. Eschbach, R.G. Hoellrich, M.J., Montfort, D.L. Chamberland, Assessment of resorbable bioactive material for grafting of critical-size cancellous defects, *J. Orthop. Res.*, 2000 Jan, 18 (1): 140-8 10716290
- [55] L.L. Hench, Bioactive Glasses: Gene Expression, An Introduction to Bioceramics, 2nd edition, L.L. Hench, ed, Imperial College Press, London, (2013), chapter 4, 71-86
- [56] R.C. Bielby, I.S. Christodoulou and R.S. Pryce *et al.*, (2004) Time and concentration-dependant effects of dissolution products of 58S sol-gel bioactive glass on proliferation and differentiation of murine and human osteoblasts, *Tissue Eng.* 10, 1018-1026.

- [57] I. Christodoulou, L.D.K. Buttery and P. Saravanapavan *et al.*, (2005), Characterization of human fetal osteoblasts by microarray analysis following stimulation with 58S bioactive gel-glass ionic dissolution products, *J. Biomed. Mater. Res.* 77B, 431-446.
- [58] A. Hoppe, N.S. Gldal, A.R. Boccaccini, A review of the biological response to ionic dissolution products from bioactive glasses and glass-ceramics, *Biomaterials* 32 (2011), 2757-2774.
- [59] R.M. Day, A.R. Boccaccini, S. Shurey, J.A. Roether, A. Forbes, L.L. Hench, S.M. Gabe, Assessment of polyglycolic acid mesh and bioactive glass for soft-tissue engineering scaffolds, *Biomaterials* 25 (2004), 5857-5866.