Lessons in Nanotechnology from
Traditional and Advanced Ceramics
J.-F. Baumard (Editor)

THE STORY OF BIOGLASS

Larry HENCH
Imperial College, London, UK

The story of Bioglass® can be considered as a small chapter in the story of mankind. A chapter of how, perhaps, skeletal creatures left the buoyancy of the seas to explore land and gradually evolved to become working, thinking, dreaming human beings. To read these implications into the story requires accepting, or at least considering three scientific principles as a starting point.

First man neither creates nor destroys matter. He only manipulates and transforms matter. The use of heat to transform clay into ceramics also transformed human culture. The invention of ceramics was a critical step in the irreversible transformation of short-lived hunter-gatherers into aging agrarians. However, the phase transitions that occur when a pot is fired are ultimately reversible. The loss of water, CO_2 or other volatiles from minerals during firing, is not permanent. The molecular elements still exist and the original inorganic phases can be reconstituted. Inorganic phase transformations are reversible.

The second starting principle lies in recognising that the creation of life is an *irreversible* phase transformation. At the onset of life, be it a single celled bacterium or a human being, the capacity exists for self-maintenance, self - replication and self - adaptation; i.e., mutation. These three distinguishing characteristics exist throughout life. But, at the time of death all three are lost, permanently and irreversibly. The chemical elements in a cell remain the same, the molecules are the same, and even the proteins and DNA often remain unaltered. But when the so-called "life force" is gone it is gone forever, the transition from life to death is irreversible. In a complex organism such as a human being with two billion cells, the loss of one cell is not fatal, it happens continuously throughout life. When a critical fraction of the cells of a vital organ die, the result is fatal. It is also not reversible. Death is forever.

Our third starting principle in the prologue to this story is perhaps more of a philosophical perspective. I suggest that as we explore the world we do not discover; we uncover. Our understanding of nature lies in uncovering what already exists. Watson and Crick did not discover the structure of DNA and the key to life, they uncovered a molecular structure and principle of replication of life that was created billions of years ago. The process of creation

of DNA and the origin of life is still not known 50 years after Watson and Crick's paper, and may never be known. To uncover a principle or a phenomenon does not necessarily mean to understand its origins. Answering "How?" does not necessarily lead to answering "Why?"

My justification for this prologue is that the Story of Bioglass® has been a quest to answer two fundamental questions. The phenomenon that living bone can form a mechanically strong bond to a man-made material, Bioglass®, was found in 1969. The human body's defence mechanisms against foreign objects, evolved over millions of years, were not activated when the special composition of 45S5 Bioglass® (Table 1) was implanted into the femurs of rats by an orthopaedic surgeon, T.K. Greenlee, Jr. Six weeks after implanting the samples, Dr. Greenlee called me to report, *"Larry, what are those ceramics you gave me? They won't come out of the bone. I've pulled them, I've hit them. They won't come out. They are bonded to the bone!"*

TABLE 1 - Composition and properties of bioactive glasses and glass ceramics used clinically.

Composition (wt%)	**45S5 Bioglass**	**S53P4 (Abmin Dent1)**	**AW Glass Ceramics (Cerabone)**
Na_2O	24.5	23	0
CaO	24.5	20	44.7
CaF_2	0	0	0.5
MgO	0	0	4.6
P_2O_5	6	4	16.2
SiO_2	45	53	34

THE FIRST QUESTION: "HOW?"

This finding led to the first question in the story of Bioglass®. *"How does a man-made material form a bond to living tissue?"* The research to answer this took fifteen years and involved the creative works of dozens of former students and colleagues. The answer is now reasonably well known, as documented in numerous books and reviews. Only a very brief synopsis will be repeated in this article. Instead, the focus of this Story of Bioglass® will be on a much more difficult question, *"Why?"*

THE SECOND QUESTION: "WHY?"

During the exploration of "How does Bioglass® bond to bone?" an exciting new phenomenon was uncovered. Bioglass® not only bonded to existing bone but also stimulated the formation of *new* bone. This new process was given a new name, *osteoproduction*, by Dr. June Wilson Hench who was the first person to document the finding. She observed that when Dr. Sam Low, Professor of Periodontology implanted particles of Bioglass in a bone defect in the jaws of monkeys that three things happened: First, new bone grew quickly along the surface of the particles from host bone, a process called *osteoconduction*. Secondly, new soft tissue fibers, the periodontal membrane, grew across the top of the defect, reconnecting bone tissue to the tooth. Third, new bone formed as bridges between Bioglass® particles throughout the defect, i.e., osteoproduction. The hole in the bone was regenerated. A key question arose,

"WHY DOES BIOGLASS LEAD TO BONE REGENERATION?"

The first clues to the answer began to appear at University of Florida, early in our quest for, "How does Bioglass® form a bond to bone?" The final answer has required eight additional years of searching at Imperial College London. The answer is now at hand. The implications of the answers are profound. They extend from the first steps of our evolution to the future of our species. Let the story begin.

THE BEGINNING

A small trip, a big step. While on a bus ride to an Army Material Conference in Sagamore, New York, July 1967, a U.S. Army Colonel, sitting next to me, told me of the devastating damage to service men in battle. He said, *"The problem is that we can save lives but not limbs. The human body rejects all the metals and plastics we use. Why don't you make a ceramic bone that will not be rejected?"* Upon returning home I discussed this suggestion with three people. Ray Splinter, then a medical student, later, a distinguished reconstructive surgeon, suggested that two orthopaedic surgeons, Dr. Bill Allen and Dr. Ted Greenlee, might be interested. A proposal to the U.S. Army Medical R&D Command resulted from our discussions.

A one year contract for $85,000 was funded in October 1969. The Phase Diagrams for Ceramics Na_2O-CaO-SiO_2 system was used to design the first glass compositions. I selected 45 weight % $Si0_2$ as the network former for four reasons:

1) It was close to a ternary eutectic with a low melting point making the glass easy to melt in a standard furnace with SiC heating elements
2) A large, 24.5 weight %, amount of CaO could be incorporated as a glass network modifier; having learned from Ham's *Histology* that bone contained substantial amounts of hydroxyapatite (HA) bone mineral, a hydrated salt of Ca and P ions.
3) 24.5% Na_2O could be used as a flux, having also learned that all body fluids contain large amounts of Na ions, and
4) 6 weight % of P_2O_5 could be used as a mixed valence flux and network former and also provide phosphate ions to react with calcium ions to form bone mineral.

Thus was 45S5 Bioglass® formulated, 45S for the percentage of network former SiO_2 and 5 for the atomic weight ratio of Ca/P in the glass. Many formulations have been tested over time but the first was the best[1-2]. The first implants were small polished glass rectangles, approximately 0.5cm x 1cm x 0.2cm. As Dr. Greenlee reported, "They bond to bone." No glass or ceramic composition bonds faster. When the percentage of silica in the glass is increased to 60 weight percent, bone bonding no longer occurs. This bone-bonding boundary[3-11] is shown in Figure 1. Glass compositions within the boundary are now known as bioactive glasses. Compositions in the centre, extending to 55 weight percent SiO_2, are known to possess class A bioactivity with rapid bone bonding. Compositions between 45 and 55 w/o SiO_2 also have the property of bonding to soft connective tissues, as found by Dr. June Wilson Hench when she evaluated 45S5 Bioglass® as subcutaneous (under the skin) implants in rabbits and sheep.[12]

THE BIOACTIVE BOUNDARY

The compositional diagram in Figure 1 is not a phase equilibrium diagram. It is a kinetics diagram. The boundaries of bioactivity are affected by any variable that influences the rate of bonding of bone or soft tissues to an implant of a specific composition. Human bone bonds more slowly than rat bone so the compositional limits are more restricted for bioactive glasses used clinically.[14-20]

Increasing the surface area of glasses by making them into powders, such as the commercial products Perioglas® and NovaBone®, accelerates surface reaction kinetics and expands the boundary[21-22]. Changing processing methods, such as use of sol-gel processing, results in gel-glasses with nanometer-sized pores and thereby alters the kinetics and bioactive boundaries considerably[23-24]. Gel-glasses composed of CaO and SiO_2 are bioactive when 90% SiO_2 and only 10% CaO are present. The bioactivity of 70/30 (70mol%

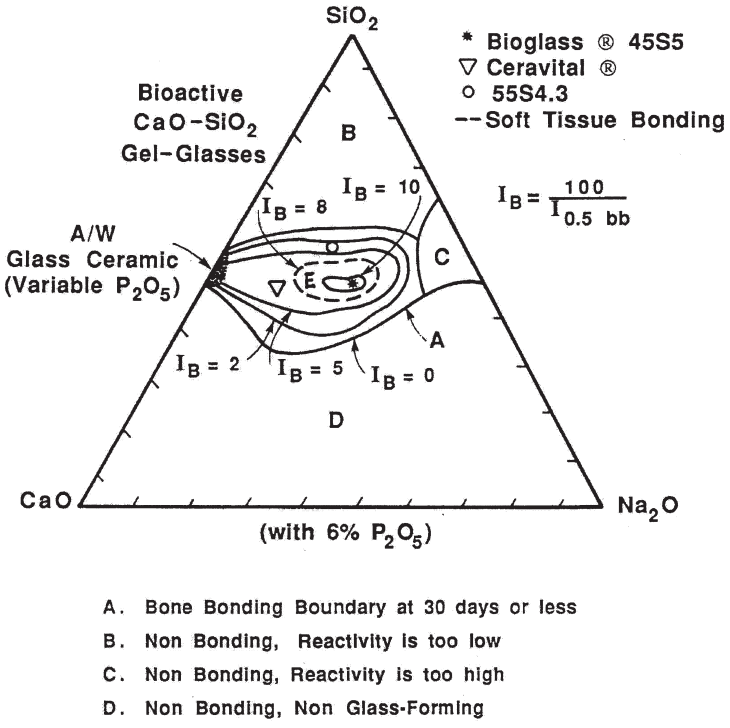

FIGURE 1

SiO_2 – 30 mol% CaO) gel-glasses is equivalent to that of 45S5 melt-derived glass, as indicated in the brackets in Figure 1.

HOW DOES BIOGLASS BOND TO BONE?

Kinetics provide the answer. Twelve reactions stages are involved. The first five stages occur at the surface of the glass with the sequence beginning by the fast ion exchange of sodium ions for hydrogen and hydronium ions in body fluids (Figure 2).[5,7,9,23] A large concentration of silanols (Si-OH) is formed on the surface by the cation exchange followed rapidly by a condensation reaction (2SiOH) – (H_2O) = (Si –O-Si) to form siloxane bonds and a silica-rich gel surface. Network dissolution also occurs releasing soluble silica as well as calcia and phosphate ions to the interfacial body fluids. The hydrated calcia ions precipitate in the mesoporosity of the silica gel and react with phosphate and carbonate ions to nucleate a hydroxycarbonate apatite (HCA) layer on the surface. The HCA is mineralogically equivalent to bone mineral and is equally biologically active.

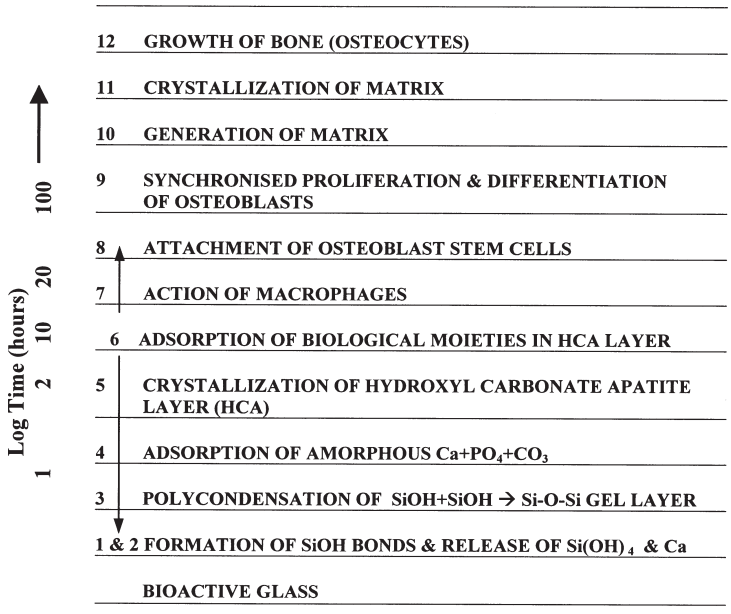

FIGURE 2

Cells rapidly attach to the growing HCA layer and begin to multiply and change (differentiate) into bone growing cells (*osteoblasts*). These highly specialised bone cells produce a unique protein, called Type 1 collagen, which nucleates HCA crystal platelets within the collagen fibrils. The bone growing cells become encased in the HCA reinforced collagen layer and change into a mature bone cell, called on *osteocyte*, which is no longer capable of dividing. An osteocyte lives a long time, usually many years, fed by nutrients obtained by diffusion from small capillaries.

The kinetics of the first five stages of the glass surface reactions determine whether the following seven stages precede to completion. If the rates are too rapid the glass dissolves and is toxic to the cells. If the rates are too slow new bone cells do not grow. Instead, the interface develops a layer of fibroblast cells characteristics of scar tissue; the collagen in the tissue is not capable of becoming mineralised and the interface is non - bonded.

Bone bonding only occurs when the rates of the glass surface reactions are synchronised with cell cycles, as discussed below. Bioactive, bone – bonding glasses have optimal reaction rates that match the rates of cell proliferation and differentiation. This is the answer to "How does Bioglass bond to bone?"

"WHY DOES BIOGLASS REGENERATE BONE?"

Kinetics also provide the first clue to understanding bone regeneration. The remaining clues come from cell biology. The key stage of kinetics is the rate of network dissolution. Network dissolution releases soluble silica and calcia ions from the glass surface. For fifteen years this consequence of ionic dissolution was largely ignored as research concentrated on how interfacial bonds were created. The emphasis was on formation of the HCA layer. Numerous reviews, including my own, attributed rate of HCA layer formation to be the feature that distinguished bioactive ceramics, glasses and composites from bio - inert materials.

June Wilson's identification of osteoproduction in periodontal defects in monkeys containing 45S5 bioactive glass particles was the first indication that something more fundamental than HCA layer formation was occurring in bone regeneration[21]. A series of studies led by Dr. H. Oonishi, a leading orthopaedic surgeon, in Osaka, Japan, provided the second set of clues[25]. The Oonishi animal model is a critical size hole, in a rabbit femur; the hole is not filled by the natural bone repair.

FIGURE 3

These studies showed that loosely filling the hole with 45S5 Bioglass® particles led to rapid bone repair. Regeneration of trabecular bone, the normal type of bone in this location of the femur, started as early as two to five days. This was a remarkable finding. Figure 3, courtesy of Dr. Oonishi, is a scanning electron micrograph back scattered electron image that shows extensive bridges of newly formed bone between the Bioglass particles. The bone bridges occur first between the corners of the particles and then grow laterally. It took several years to recognise the significance of this observation.

In the meantime several critical experiments were conducted at Imperial College London in Professor Julia Polak's laboratories, an integral part of what is now the Imperial College Tissue Engineering and Regenerative Medicine Centre. These experiments were developed by Professor Polak and myself to test a hypothesis and conducted by a brilliant doctoral student Ionnis Xynos, with bone biology advice from Dr. Lee Buttery and molecular biology supervision by Dr. Alasdair Edgar. The hypothesis for the research was: Hypothesis for Class A Bioactive Materials

Intracellular effects: enhanced differentiation and proliferation of bone stem cells via gene activation and

Extracellular effects: adsorption and desorption of growth factors without loss of conformation and biological activity.

This hypothesis was not conceived *de nouvo*. It originated from recognising the potential relationship of several disparate findings over 30 years by researchers in different disciplines. Details are given in reviews by Hench, Lobel and West,[23] so I only summarise them here.

More than thirty years ago, Dr. Edith Carlisle, a researcher in nutrition, found that silicon was a critical trace element in the body. She found that when hydrated silicon was absent bones do not mineralise; they remained as cartilaginous structures. Her work in chickens was quickly confirmed in mammals by Schwarz and Milne. Silicon was found to be concentrated in the mineralization front of newly forming long bones.

A second finding leading to our hypothesis was in a paper published by Keeting *et al*, in 1992[26]. They grew cultures of human bone cells and exposed them to various concentrations of the dissolution products of soda zeolites. The bone cells responded favourably to the mixture of soluble sodium, silica and aluminate ions and generated enhanced concentrations of growth proteins, especially a growth factor called TGF-beta. Their study also showed that soluble silica alone, as silicic acid, had a positive effect on the bone cell cultures.

Investigations of the mechanisms of biosilicification, such as growth of the silica exoskeleton of diatoms and radiolarians and silica - rich plants such as equisetum, reviewed at a CIBA Foundation Conference on Biochemistry

of Silica,[27] seemed to converge towards our hypothesis that soluble silica and calcia affects living cells at a genetic level. Semi-empirical quantum mechanics based models calculated by Dr. Jon West, Keith Lobel and myself confirmed the favourable energetics of biogenic silica reaction pathways[28-29]. Two clues from our very early attempts to understand mechanisms of Bioglass® bonding reinforced the scientific basis for the hypothesis.

A paper by Bruce Hartwig and myself[30] demonstrated the strong empirical binding of simple amino acids to hydroxylated silica bonds. This simple model system provided insight into a later study by Seitz *et al*, where it was shown that the life cycle of cells could be greatly affected by the attachment of their cell membrane proteins to a bioactive surface[31].

TESTING THE HYPOTHESIS: CONTROL OF OSTEOBLAST CELL CYCLE

A significant implication of the body of work reviewed above was that the biologically active surface of Bioglass®, or its dissolution products, the soluble silica and calcia ions, could be affecting the life of cycle of cells. In order to understand this phenomenon it is necessary to consider what alternatives are possible in the life of a single bone cell.[32] As mentioned above, after a bone cell is encased in a mineralised collagen matrix it usually lives a long time, as an osteocyte. If an osteocyte is damaged, such as when a bone breaks, it cannot repair itself. The mineralised matrix must be dissolved and the dead bone cell broken down by a scavenging cell called an *osteoclast*. Capillaries bring new bone stem cells to the site of repair.

One of three fates await the bone repair cells, as illustrated in Figure 4. If the biochemical and biomechanical environment is favourable the cell will pass from its normal growth phase, termed G, into a phase where its DNA is replicated (called the synthesis or S phase). A number of proteins exist in human cells that ensure that the cell is able to duplicate its chromosomes correctly. These comprise the G/S checkpoint shown in Figure 4. If the checkpoint is not passed the cell is switched on to programmed cell death, called apoptosis, also shown in Figure 4.

The experiments performed by Xynos *et al* showed that a large fraction of bone cells originally in a cell culture were switched into apoptosis when exposed to either a 4555 Bioglass® substrate or the soluble ionic dissolution products of Bioglass® [32-35]. This was an especially important finding because the remaining cells were able to pass through the S phase of their cell cycle and eventually start to multiply by cell division, called mitosis. Two daughter cells, shown in Figure 4, result from cell division, each with equivalent DNA. When the biochemical and biomechanical stimuli are sufficient the genes of a daughter cell are switched on to produce several families of proteins that

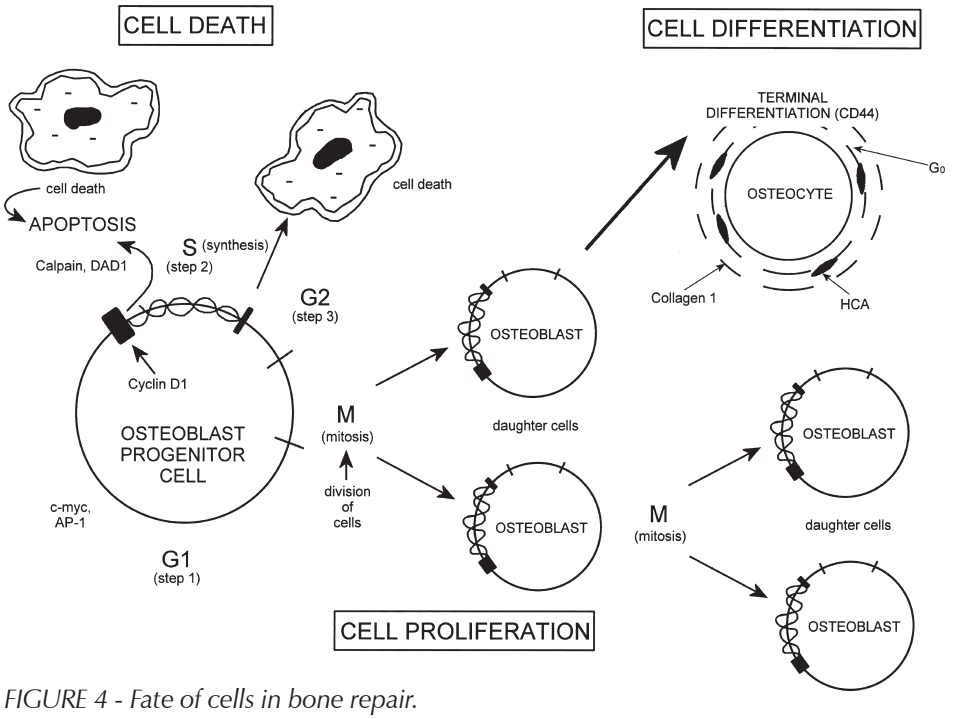

FIGURE 4 - Fate of cells in bone repair.

lead to production of a mineralised extracellular matrix. A new osteocyte is formed and a small segment of bone is regenerated.

Often a larger segment of new bone is needed to satisfy biomechanical requirements, such as formation of the new trabeculae in Figure 3. A fraction of the daughter cells must not become osteocytes. Instead, they must continue to multiply by cell division until all the regenerated bone segments are connected together. The biochemical gradients of soluble Si and Ca released by the slow, controlled dissolution 4555 Bioglass® particles provides a favourable environment for both bone cell division and bone cell differentiation. The selective process starts as early as 48 hours and is completed within a few days. In clinical conditions bone regeneration can occur as quickly as three months or take as long as nine months depending upon the size and location of the bone defect, age and health of the patient.

GENE ACTIVATION

A critical feature of our hypothesis was that genetic control of the osteoblast cell cycle is influenced by ions released from Bioglass®. Several molecular biology techniques were used by Xynos *et al* to test this hypothesis[36-37]. Gene

micro arrays showed that exposure of primary human osteoblasts, obtained from excised femoral heads, to bioactive ions activated seven families of genes. There was a 200 to 700% increase in the expression of these genes over those of control cultures with the same source of cells.

The activated genes include transcription factors and cell cycle regulators which are essential for osteoblasts to commence cell division. DNA repair proteins were also upregulated by exposure of osteoblasts to the bioactive Si and Ca ions, as were genes controlling apoptosis. The newly divided cells began to synthesize proteins that are potent growth factors and cytokines (cell membrane proteins) that are characteristic of the mature osteocyte phenotype. The most abundant growth factor in bone, insulin-like growth factor II (1GF – II), was increased by 320% when the bioactive stimuli were present. Numerous genes that express extracellular matrix components were also stimulated by the bioactive ions. A specific phenotype marker of osteocytic differentiation is a protein termed CD44. The soluble bioactive ions enhanced expression of the CD44 gene by 700% over control cell cultures.

IMPLICATIONS FOR THE PAST

The prologue suggests that our understanding of the story of Bioglass® might provide us clues about the origin of our species. We have learned that many genes of cells capable of regenerating mineralised bone are activated when exposed to critical concentrations of soluble silicon and calcium ions. Recent findings from Beilby *et al* in Professor Polak's laboratory show that embryonic stem (ES) cells are sensitive to the soluble bioactive ions [38]. When the critical concentrations of soluble silicon and calcium are present the genetic code switches ES cells to become osteoblasts that produce many types of proteins that result in mineralised bone. This finding suggests that the seminal mutation that occurred approximately four hundred million years ago when cartilaginous fish began to form mineralised bone was initiated by ingestion of species containing large amounts of biologically fixed silicon.

Diatoms and radiolarians form microscopic exoskeletons composed of hydrated silica grown on a protein template[28, 29]. A fish diet composed primarily of such organisms could have led to the genetic mutations that are characteristic of cells of the osteoblast lineage. Such mutations have enhanced the survivability of the newly emerging species of fish. Mineralization would lead to strengthening of the skeleton. A stronger skeleton would support the head and make it possible for fish to feed above the air – water interface where gravity must be overcome. Higher oxygen content of the air and greater density of food would have provided a favourable environment for the fish with mineralised skeletons and ensure evolutionary survival of species with the favourable mutation.

The advantages of the mineralised skeleton led to many new species of fish and evolution of fins capable of walking on the shore. The mutual genetic advantages of breathing oxygen and a strong mineralised skeleton led to a genetic explosion of species including: more than twenty thousand bony fishes, amphibians, reptiles and eventually mammals.

Thus, the biogenic fixation of soluble silica weathered from the inorganic minerals of the geosphere appears to be intimately associated with creating the genetic diversity and complexity of the biosphere.

IMPLICATIONS FOR THE FUTURE

Human beings are enormously complex biological creatures. We are composed of many billions of cells, each at some stage, containing the DNA to replicate the entire organism. Our DNA is folded into twenty one chromosomes which contain approximately sixty thousand genes. Large segments of DNA no longer appear to have any function in maintaining life. They are referred to as "junk DNA". Most genes in a cell are also unused most of the time. However, when cellular repair is required the correct sequence of gene expression is necessary. As we age, fewer cells are available that can start the repair process. As a consequence all of our organs and our skeleton slowly deteriorate.

Our findings that critical concentrations of soluble silica and calcia activate genes that initiate cellular proliferation and differentiation has profound implications. If we can learn how to deliver these ions on a daily basis through our diet or by food supplements we may be able to slow down aging of our connective tissues.

Modern technology has made it feasible for most of us to increase our length of life. Findings from the Story of Bioglass® may make it possible to increase our quality of life in our later years. This might seem to be an impossible goal. However, we must remember that only thirty – five years ago materials that bonded to living tissues seemed to be an impossible goal.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the collaborations with Professor Dame Julia Polak and financial support of the UK Engineering and Physics Research Council and Medical Research Council.

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], 117-141 (1971).
2. C.A. Beckham, T.K. Greenlee, Jr. and A.R. Crebo, "Bone Formation at a Ceramic Implant Interface", Calc. Tiss. Res., 8, 165-171, (1971).
3. L.L. Hench, "Biomaterials", *Science* 208, 826 (1980).
4. L.L. Hench and J.W. Wilson, "Surface-Active Biomaterials," Science, 226 630 (1984).
5. L.L. Hench, *J. Am. Ceram. Soc.* 81, 1705 (1998).
6. J. Wilson, G.H. Pigott, F.J. Schoen, and L.L. Hench, "Toxicology and Biocompatibility of Bioglass," J. Biomed. Mater. Res., 15 805 (1981).
7. T. Yamamuro, L.L. Hench, and J. Wilson, eds., *Handbook on Bioactive Ceramics: Bioactive Glasses and Glass-Ceramics, Vol. I*. CRC Press, Boca Raton, FL, 1990.
8. S.F. Hulbert, J.C. Bokros, L.L. Hench, J. Wilson, and G. Heimke, "Ceramics in Clinical Applications, Past, Present and Future"; pp. 189-213 in *High Tech Ceramics*. Edited by P. Vincenzini. Elsevier Science Pub. B.V., Amsterdam, (1987).
9. L.L. Hench and June Wilson, *An Introduction to Bioceramics*, World Scientific, London, (1993).
10. U. Gross, R. Kinne, H.J. Schmitz, V. Strunz, "The Response of Bone to Surface Active Glass/Glass-Ceramics," *CRC Critical Reviews in Biocompatibility*, D. Williams, ed. 4 2 (1988).
11. U. Gross and V. Strunz, "The Interface of Various Glasses and Glass-Ceramics with a Bony Implantation Bed," J. Biomed. Mater. Res., 19 251 (1985).
12. June Wilson and D. Nolletti, "Bonding of Soft Tissues to Bioglass®," in *Handbook of Bioactive Ceramics. Vol. 1* Edited by T. Yamamuro, L.L. Hench, and J. Wilson, CRC Press, Boca Raton, FL, pp. 283-302 (1990).
13. J. Wilson, A.E. Clark, E. Douek, J. Krieger, W.K. Smith, J.S. Zamet, "Clinical Applications of Bioglass® Implants", in *Bioceramics 7,* Andersson, Happonen, Yli-Urpo (eds), Butterworth-Heinemann, Oxford, 415, (1994).
14. C.A. Shapoff, D.C. Alexander, A.E. Clark, "Clinical Use of A Bioactive Glass Particulate in The Treatment of Human Osseous Defects", Compendium Contin Educ Dent, 18, 4, 352-363 (1997).
15. G.E. Merwin, "Review of Bioactive Materials for Otologic and Maxillofacial Applications"; pp. 323-328 in *Handbook of Bioactive Ceramics, Vol I*. Edited by T. Yamamuro, L.L. Hench and J. Wilson. CRC Press, Boca Raton, FL, 1990.
16. E. Douek, "Otologic Applications of Bioglass® Implants," in *Proceedings of IVth International Symposium on Bioceramics in Medicine*. Edited by

W. Bonfield, London, Sept. 10-11, (1991).
17. K. Lobel, "Ossicular Replacement Prostheses", *in Clinical Performance of Skeletal Prostheses,* Hench and Wilson (eds), Chapman and Hall, Ltd, London, pp 214-236 (1996).
18. H.R. Stanley, M.B. Hall, A.E. Clark, J.C. King, L.L. Hench, and J.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, 95-105 (1997).
19. H.R. Stanley, A.E. Clark, L.L. Hench, "Alveolar Ridge Maintenance Implants", in *Clinical Performance of Skeletal Prostheses*, L.L. Hench and J Wislon, eds, Chapman and Hall, Ltd., London, pp 255-270, 1996.
20. L.L. Hench, J. Wilson, Eds., *Clinical Performance of Skeletal Prostheses,* Chapman & Hall, London, chaps. 13 and 15 (1996).
21. J. Wilson and S.B. Low, "Bioactive Ceramics for Periodontal Treatment: Comparative Studies in the Patus Monkey", J. Appl. Biomaterials, Vol 3, 123-169 (1992).
22. A.E. Fetner, M.S. Hartigan, S.B.Low, "Periodontal Repair Using Perioglas® in NonHuman Primates: Clinical and Histologic Observations", Compendium Contin Educ Dent, 15 (7), 932-939, (1994).
23. L.L. Hench and J.K. West, "Biological Applications of Bioactive Glasses", Life Chemistry Reports, Vol. 13, 187-241 (1996).
24. L.L. Hench, D.L. Wheeler and D.C. Greenspan, "Molecular Control of Bioactivity in Sol-Gel Glasses", J. Sol-Gel Sci. and Tech. 13, 245-250 (1998).
25. 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 different particle sizes." J. Biomed. Mater. Res., Vol 44 (1) 31-43 (1999).
26. P.E. Keeting, M.J. Oursler, K.E. Wiegand, S.K. Bonds, T.C. Spelsberg and B.L. Riggs, "Zeolite A increases proliferation, differentiation and transforming growth factor b production in normal adult human osteoblast-like cells *in-vitro*," *J. Bone and Mineral Res*, 7, 1281 – 89 (1992).
27. D.E. Evered and M. O' Connor, eds *Silicon Biochemistry*, Ciba Foundation, Symposium 121, J. Wiley & Sons, NY (1986).
28. K.D. Lobel, J.K. West and L.L. Hench, "A Computational Model for Protein-mediated Biomineralization of the Diatom Frustule," *Marine Biology*, 126, 353-360 (1996).
29. K.D. Lobel, J.K. West and L.L. Hench, "Computer Models of Molecular Structure of Biomaterials," in *Computer Technology in Biomaterials Science and Engineering*, J. Vander Sloten, ed., J. Wiley pp 45-88 (2000).
30. B. Hartwig and L.L. Hench, "Epitaxy of Poly-L-Alanine on a-Quartz and Glass-Ceramic Surfaces," *J. Biomed. Materials Research* 6 [5] 413-424 (1972).

31. T.L. Seitz, K.D. Noonan, L.L. Hench and N.E. Noonan, "Effect of Fibronectin on the Adhesion of an Established Cell Line to a Surface Reactive Biomaterial," *J. Biomed. Maters. Res.* 16 [3] 195-207, (1982).
32. L.L. Hench, J.M. Polak, I.D. Xynos, L.D.K Buttery, "Bioactive Materials to Control Cell Cycle," *Mat Res Innovat 3*, 313-323 (2000).
33. I.D. Xynos, M.V.J. Hukkanen, J.J. Batten, L.D. Buttery, L.L. Hench, J.M. Polak, "Bioglass® 45S5 Stimulates Osteoblast Turnover and Enhances Bone Formation *In Vitro*: Implications and Applications for Bone Tissue Engineering," *Calc Tiss Int* 67, 321-329 (2000).
34. I.D. Xynos, A.J. Edgar, L.D. Buttery, L.L. Hench, J.M. Polak, "Ionic Dissolution Products of Bioactive Glass Increase Proliferation of Human Osteoblasts and Induced Insulin-like Growth Factor II mRNA Expression and Protein Synthesis," *Biochem. Biophys. Res. Comm*. 276, 461-465 (2000).
35. H. Oonishi, L.L. Hench, J. Wilson, F. Sugihara, E. Tsuji, M. Matsuwura, S. Kin, T. Yamamoto, S. Mizokawa, "Quantitative Comparison of Bone Growth Behaviour in Granules in Bioglass®, A-W Glass Ceramic, and Hydroxyapatite", *J Biomed Mater Res* 51, 37-46 (2000).
36. 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).
37. L.L. Hench, "Glass and Genes: The 2001 W.E.S. Turner Memorial Lecture," *Glass Technology*, 44, 1 1-10 (2003).
38. R.C. Beilby, R.S. Pryce, L.L. Hench, and J.M. Polak, "Enhanced derivation of osteogenic cells from murine ES cells following treatment with ionic dissolution products of 58S sol-gel glass," *Tissue Engineering* (2004).