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Bioactive materials for tissue engineering, regeneration and repair

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Tissue engineering is an interdisciplinary field which applies the principles of engineering and the life sciences to the design, construction, modification, growth and maintenance of living tissues [1, 2]. One of two approaches can be taken: (1) *in vitro* construction of bioartificial tissues from cells seeded onto a resorbable scaffold or (2) *in vivo* modification of cell growth and function to stimulate tissue regeneration [2, 3]. This concept represents a shift in emphasis from replacement to regeneration of diseased or damaged tissues, in which the development of bioactive materials has played a significant role.

This paper will begin with an overview of the use of biomaterials as implants and their limitations, leading to the reasons for the dramatic shift in focus regarding the approach to repairing damaged tissues. The majority of the paper will discuss the ways in which biomaterials can be developed to implement the concept of tissue engineering. Finally, the implications of these developments for future treatment of damaged or diseased tissues will be considered.

1. A new generation of biomaterials

The first generation of biomedical materials used within the body was largely biologically inert, or nearly-inert. The goal at the time was to "achieve a suitable combination of physical properties to match those of the replaced tissue with a minimal toxic response in the host" [4]. It was once thought that all materials, when placed inside the body, would elicit a foreign body response, the formation of a non-adherent fibrous capsule around the implant. However the discovery, in 1969, of a four-component glass which could bond to living tissue showed that it is possible for certain materials to elicit a controlled action and reaction in the physiological environment. By the mid-1980s bioactive materials had reached clinical use in a variety of orthopaedic and dental applications, including various compositions of bioactive glasses, ceramics, glass-ceramics and composites, as shown in Table I. Another advance in this second generation of materials for medicine was the development of resorbable biomaterials, designed to break down chemically and be resorbed at an equivalent rate to tissue regrowth. Ultimately the foreign material is replaced by regenerating tissue and the implant site becomes virtually indistinguishable from the host tissue. An example of this is the biodegradable suture, in which the polymer composed of polylactic (PLA) and polyglycolic (PGA) acids decomposes and metabolises into CO_2 and H_2O.

Developments throughout the last century, such as drugs, vaccines, water treatment and improved hygiene have resulted in a vast increase in the average life expectancy in developed countries. While the clinical success of bioinert, bioactive and resorbable implants has greatly improved the quality of life for tens of millions of people, research [6–8] shows that a third to half of prostheses fail within 10–25 years and patients require revision surgery. Twenty years of research has had only small effects on failure rates [6]. In fact the improvement of first- and second-generation materials is limited as all man-made biomaterials used for the repair or restoration of the body represent a compromise [4]. Synthetic materials cannot respond to changing physiological loads or biochemical stimuli, unlike living tissues. This limits the lifetime of artificial body parts. Thus there is need to consider a shift towards a more biologically based method for the repair and regeneration of tissues.

The new challenge in biomaterials is to enhance the body's own regenerative capacity by stimulating genes which initiate repair at the site of damage or disease. A third generation of biomaterials is being developed to do this. The separate concepts of bioactive and resorbable materials have been combined to make bioactive materials resorbable. Third generation bioactive glasses and macroporous foams are being designed to activate genes that stimulate regeneration of living tissues. Molecular modifications of resorbable polymer systems elicit specific cellular responses. These tailored materials make available the two approaches to tissue engineering, described above.

Tissue-engineered constructs can be produced by seeding progenitor cells onto modified resorbable scaffolds. The cells grow outside the body, become differentiated and mimic naturally occurring tissues. The construct can then be implanted into a patient. In time the scaffold is resorbed and replaced by host tissue that

TABLE I Clinical uses of biomaterials [5]

Application	Materials[a]
Orthopaedic	Al_2O_3
	Stabilised ZrO_2
	HA powders
	Bioactive glass powders
Coatings for bioactive bonding	HA
	Bioactive glass-ceramics
Bone space fillers	Tricalcium phosphate
	Calcium phosphate salts
Dental implants	Al_2O_3
	HA
	Bioactive glasses
Artificial tendon and ligament	PLA-carbon-fibre composite
Periodontal pocket obliteration	HA
	HA-PLA composite
	Tricalcium phosphate
	Calcium phosphate salts
	Bioactive glasses
Alveolar ridge augmentation	HA
	HA-autogenous bone composite
	Bioactive glasses
Maxillofacial reconstruction	Al_2O_3
	HA
	HA-PE composite
	Bioactive glasses
Spinal surgery	Bioactive glass-ceramics
	HA
Therapeutic treatment of tumours	Rare-earth-doped aluminosilicate glasses
Artificial heart valves	Pyrolytic carbon coating
Otolaryngological	Al_2O_3
	HA
	Bioactive glasses
	Bioactive glass-ceramics
	HA-PE composite

[a]HA is hydroxyapatite, PE is polyethylene and PLA is poly (lactic acid).

includes a viable blood supply and nerves. The living construct will adapt to the physiological environment and should provide long-lasting repair.

Biomaterials can be used *in situ* in the form of powders, solutions or doped microparticles to stimulate local tissue repair. The 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, which 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 required tissues *in situ*. For example, when a particulate bioactive glass is used to fill a bone defect there is rapid regeneration of bone that matches the architecture and mechanical properties of bone at the site of repair.

2. Bioactive glasses

2.1. The discovery

In 1969 Hench and colleagues at the University of Florida synthesised a special composition of melt derived soda-lime-phosphate-silicate glass and implanted it into the femurs of rats [9–11]. The glass did not form interfacial scar tissue, but bonded to the living bone and could not be removed from the implant site. The glass was an invert glass containing only 45 wt% SiO_2, with network modifiers of 24.5 wt% Na_2O and 24.5 wt% CaO, called Bioglass® 45S5. In addition 6 wt% P_2O_5 was added to simulate the Ca/P constituents of hydroxyapatite, the inorganic mineral phase of bone. This composition of Bioglass® is now available commercially in particulate form under the names Perioglass® and Novabone® and its clinical use includes bone grafting in orthopaedic, dental, maxillofacial and otolaryngological applications.

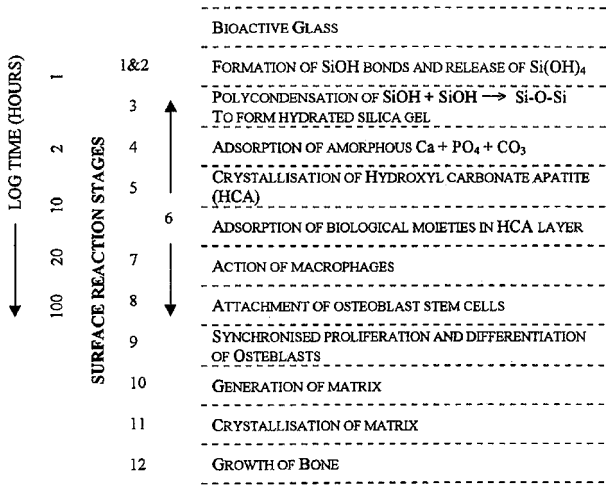
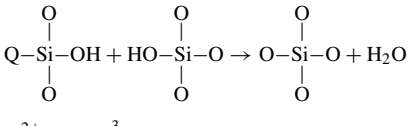


Figure 1 Sequence of interfacial reactions involved in forming a bond between bone and a bioactive glass [12].

On exposure to aqueous solution bioactive glasses undergo structural and chemical changes at their surface. There are twelve reaction stages involved in the complete bonding of a glass to bone [12, 13], summarised in Fig. 1.

The first five reaction stages result in the formation of a crystalline hydroxycarbonate apatite (HCA) layer on the surface of the glass and are summarised in more detail in Table II.

TABLE II Reaction stages of a bioactive implant [12]

Stage
1 Rapid exchange of Na^+ or K^+ with H^+ or H_3O^+ from solution: $Si{-}O{-}Na^+ + H^+ + OH^- \rightarrow Si{-}OH + Na^+$ (Solution) + OH^- This stage is usually controlled by diffusion and exhibits a $t^{-1/2}$ dependence.
2 Loss of soluble silica in the form of $Si(OH)_4$ to the solution, resulting from breaking of Si—O—Si bonds and formation of Si—OH (silanols) at the glass solution interface: $Si{-}O{-}Si + H_2O \rightarrow Si{-}OH + OH{-}Si$ This stage is usually controlled by interfacial reaction and exhibits a $t^{1.0}$ dependence.
3 Condensation and repolymerisation of a SiO_2-rich layer on the surface depleted in alkalis and alkaline-earth cations: 
4 Migration of Ca^{2+} and PO_4^{3-} groups to the surface through the SiO_2-rich layer forming a $CaO{-}P_2O_5$-rich film on top of the SiO_2-rich layer, followed by growth of the amorphous $CaO{-}P_2O_5$-rich film by incorporation of soluble calcium and phosphates from solution.
5 Crystallisation of the amorphous $CaO{-}P_2O_5$ film by incorporation of OH^-, CO_3^{2-}, or F^- anions from solution to form a mixed hydroxyl, carbonate, fluorapatite layer.

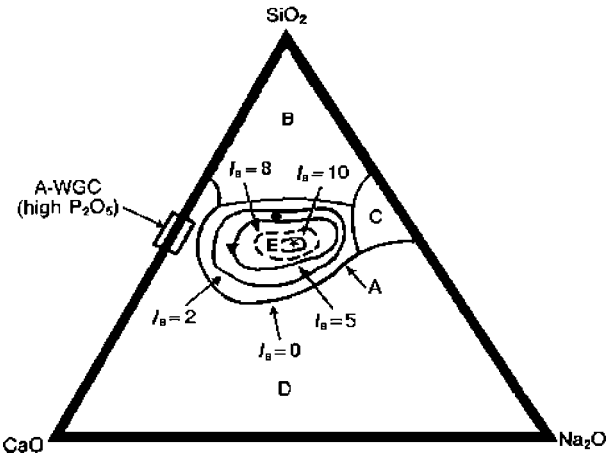


Figure 2 Compositional dependence (in wt%) of bone bonding and soft tissue bonding of bioactive glasses and glass-ceramics. All compositions in region A are bioactive and bond to bone. They have a constant 6 wt% of P_2O_5. A/W glass-ceramic has higher P_2O_5 content. Compositions in region B are bioinert and region C represents resorbable compositions. Region D is restricted by technical factors. Compositions within region E (inside the dashed line) exhibit soft tissue bonding. (*Bioglass® 5S5) [12].

The rates of these reactions vary significantly with silica content. Fig. 2 illustrates the compositional dependence of bioactivity of glasses within the SiO_2–CaO–Na_2O system, with a constant 6 wt% of P_2O_5.

All glass compositions within region A of Fig. 2 bond with bone. These are Class B bioactive materials which give rise to bone migration (osteoconduction). The first five reaction stages result in the formation of an HCA layer after a number of days. The compositions in region E of Fig. 2 exhibit rapid release of soluble ions which leads to rapid proliferation of new bone (osteoproduction) as well as bone migration. The first five reaction stages normally go to completion within 24 h. These glasses are Class A bioactive materials which bond with the collagen of soft tissue as well as bone [14, 15].

2.2. Gene activating glasses

Recent studies at Imperial College, under the direction of Professor Julia Polak, expand the understanding of reaction stages 8, 9 and 10, which are critical to the regeneration and tissue engineering of bone. Xynos *et al.* compared the effect of Class A bioactive glass (Bioglass® 45S5) with that of a bioinert control (Thermanox® plastic) on the cell cycle of human osteoblasts (hOBs) [16, 17]. The hOB cells were primary cultures obtained from excised femoral heads removed from patients aged 50–70 years, undergoing total hip replacements. Experimental procedures are given in References 16–19. Various assays were used to quantify the percentage of cells in specific segments of the cell cycle.

Fig. 3 [20] summarises the cell cycle, which is briefly described herein. Resting cells are in the G_o phase, with each new cycle beginning after the preceding mitosis. During the G1 phase (step 1 in Fig. 3) the cell grows and carries out its normal metabolism. If the local chemical environment is suitable, following a critical period of growth in the G1 phase, the cell enters

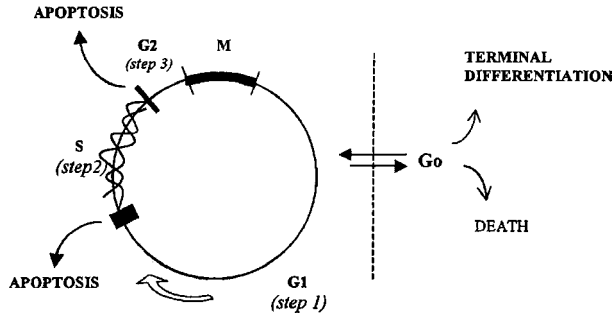


Figure 3 Schematic of cell cycle [20].

the S phase (step 2 in Fig. 3) in which DNA synthesis begins, leading to duplication of all the chromosomes in the nucleus. The cell then undergoes a second phase of growth, G2 (step 3 in Fig. 3), in which the cell prepares for its division by checking its replication accuracy using DNA repair enzymes. A critical increase in mass and activation of various growth factors is necessary for the G2-M transition and subsequent formation of two daughter cells (mitosis), essential for the formation of new bone. If the local chemical environment does not lead to full completion of either the G1 or G2 phase the cell proceeds to programmed death, apoptosis, thus eliminating the risk of multiplication of damaged genes. Bioinert and Class B bioactive materials do not provide the required local chemical environment, only Class A bioactive materials lead to rapid new bone formation *in vivo*. A consequence of these checkpoints is that as we age we produce fewer osteoprogenitor cells capable of dividing, resulting in decreasing bone density with age.

Fig. 4 [20] is a summary of the differences in surface chemical, cell biology and tissue response of Class A

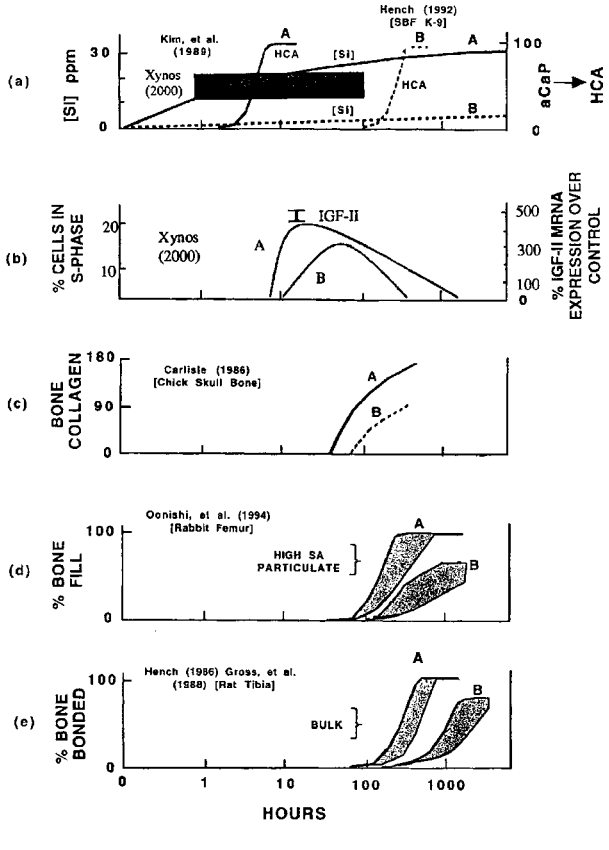


Figure 4 Time dependence of materials reactions, and cellular and tissue responses to Class A and Class B bioactive materials [20].

bioactive materials versus Class B bioactive materials. The rapid release of soluble inorganic species, especially hydrated silica ions, shown in Fig. 4a, gives rise to rapid nucleation and crystallisation of an amorphous calcium-phosphate layer to form a polycrystalline HCA layer, the high surface area of which provides many binding sites for osteoprogenitor cells. Xynos *et al.* showed that day 2 osteoblasts growing on the bioinert substrate quickly attached and entered into a cell cycle that led to the M phase and formation of a confluent sheet of cells. Such behaviour is characteristic of cells in culture that tend not to differentiate. More cells were alive than on the bioactive substrate but few of them were becoming apoptotic. In contrast osteoblasts initially grew more slowly on the bioactive glass substrate, maintaining a more compact shape with numerous dorsal membrane ruffles and microvilli, characteristic of cell activation. At 2 days twice as many cells were in the S phase and G2-M phase on the bioactive substrate and five times as many cells were apoptotic. At day 6 the proportion of osteoblasts in the S phase and especially the G2-M phase was even greater for cells grown on the bioactive material and more than seven times as many cells were being eliminated by apoptosis. More importantly there was more osteocalcin being produced by the osteoblasts grown on the bioactive material. Osteocalcin is a bone extracellular matrix non-collagenous protein produced by mature osteoblasts and its synthesis correlates with the onset of mineralisation. Scanning electron microscopy (SEM) analysis showed that osteoblasts growing on the bioactive substrate for 6 days had already self-assembled into a three-dimensional structure composed of cells and mineralised extracellular matrix, called a bone nodule, and had an organisational complexity equivalent to natural bone grown *in vivo* [16, 17].

In contrast at 6 days osteoblasts grown on bioinert substrates showed no evidence of formation of bone nodules. At day 12 more osteoblasts were present on the bioactive substrate than on the bioinert material. A significantly larger proportion of cells were in the S and G2-M phases and were organising themselves into multilayers and three-dimensional bone nodular structures. Cells incapable of differentiating into the osteoblast phenotype were still being eliminated by apoptosis at a higher rate than on the bioinert control substrate. Alkaline phosphatase synthesis, characteristic of the immature non-osteocytic phenotype, had slowed considerably for cells growing on the bioactive glass but osteocalcin production continued. The number of bone nodules growing on the bioactive substrates continued to increase and the organisation of the nodules became increasingly more complex with large number of osteocytes, terminally differentiated osteoblasts, within the nodules. At day 12 there were still no bone nodules present on the bioinert substrates although the osteoblast-like cells were still healthy.

Recent findings by Xynos *et al.* have shown that the bioactive shift of the osteoblast cell cycle described above is under genetic control [18, 19]. Within a few hours of exposure of human primary osteoblasts to the soluble chemical extracts of Bioglass® 45S5, several families of genes were activated including genes encoding nucleus transcription factors and potent growth factors, especially insulin-like growth factor (IGF-II), IGF binding proteins and proteases that cleave IGF-II from their binding proteins. IGF-II is the most abundant growth factor in bone and is a known inducer of osteoblast proliferation *in vitro*. There was a 300–500% 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 Bioglass®, as shown in Fig. 4b. It is well established that the entry of osteoblasts into the cell cycle (G_o/G1 transition) and subsequent commencement of cell division is regulated by a family of transcription factors. These specific proteins are required for a bone stem cell (osteoprogenitor) to become a bone growing cell (osteoblast). The recent results by Xynos *et al.* [18, 19] show that treating human osteoblast cultures with the ionic products of bioactive glass dissolution for 48 h activates expression of a large number of transcription factors, cell cycle regulators and genes involved in apoptosis. There was also a seven-fold increase in expression of CD44, a specific phenotype marker of osteocytic differentiation [21]. Osteocytes are terminally differentiated osteoblasts, not capable of division but responsible for synthesising and maintaining mineralised bone matrix wherein they reside [22].

The above 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. Cells incapable of forming new bone are eliminated from the cell population, a characteristic missing when osteoblasts are exposed to bioinert or Class B bioactive materials. The biological consequence of genetic control of the cell cycle of osteoblast progenitor cells is the rapid proliferation and differentiation of osteoblasts and enhanced regeneration of bone (Fig. 4d and e). The clinical consequence is rapid fill of bone defects with osteoid tissue that is capable of mineralising and becoming structurally equivalent to new bone.

2.3. Bioactive gel-glasses

Sol-gel processing is a low-temperature synthesis, in which the hydrolysis and condensation of the tetraethyl orthosilicate, calcium and phosphorous alkoxide precursors result in the formation of a dispersion of colloidal particles in a liquid, otherwise known as the sol. The subsequent condensation and polymerisation reactions turn the sol into a gel, an interconnected, rigid network with pores of submicrometre dimensions. The gel is aged, resulting in the coarsening of pores, dried to remove the pore liquid then stabilised to remove excess hydrophilic silanol groups (Si─OH) from the surface. The sol-gel process is described in more detail elsewhere [23–25].

The stages of ageing, drying and stabilisation all occur at elevated temperatures, but generally no higher than 700°C, which is much lower than the temperatures required to produce melt derived glasses. This is

just one of many advantages of sol-gel derived glasses over melt derived glasses, important for biomedical applications. Low processing temperatures as well as high silica and low alkali contents offer the potential for improved purity, required for optimal bioactivity. A homogeneous solution is achieved before polymerisation occurs, thereby reducing the presence of heterogeneities in the final glass. Better control over bioactivity can be obtained by altering the composition or microstructure. Like melt derived glasses, gel-glasses lose bioactivity with increasing SiO_2 content [26], but a wider range of bioactive compositions of gel glasses can also be used, up to 90 mol% SiO_2, whereas no melt derived glasses with more than 60 mol% SiO_2 are bioactive. The mechanism for HCA formation on bioactive gel glasses follows the same five stages as those for melt derived glasses, detailed in Table II.

The significant characteristic of sol-gel derived glasses is their inherent nanometre scale porosity. An interconnected network of mesopores (2–50 nm) is responsible for a high surface area which enables more rapid dissolution and faster HCA layer formation, i.e., enhanced bioactivity. The mesopores are proposed to act as initiation sites for the nucleation of HCA crystals [27, 28]. The porous structure is also thought to be responsible for the extended silica composition range of bioactive gel glasses. Although network break-up becomes more difficult with increasing silica content, another consequence is a larger surface area [29], which enhances ion exchange.

Sol-gel derived glasses lend themselves to the development of third generation biomaterials for tissue engineering in several ways. Gel glasses exhibit significant resorbability when their pores reach a certain size [30]. Rates of resorption can be controlled by altering the pore texture of gel-glasses [31, 32]. Gel-glasses possess the ability to be foamed into structures containing interconnected pores of 10–200 μm, mimicking the architecture of trabecular bone [33]. Bioactive resorbable scaffolds will be discussed later in this paper. During sol-gel processing the gel is completely interpenetrated by a pore liquid which consists of a highly structured hydrated layer that has similar physical and chemical properties to the bound water contained within highly hydrated connective tissues such as cartilage. Biological molecules can exchange with these hydrated layers inside the pores of gel glasses and maintain their conformation and biological activity [25, 34–36]. This allows for the impregnation of gel glasses with biologically active phases, such as growth factors. Molecular tailoring of bioactive scaffolds will be discussed later in this chapter.

3. Bioactive glass-ceramics

The clinically most important bioactive glass-ceramic is A/W glass-ceramic [37]. It is composed of apatite [$Ca_{10}(PO_4)_6(OH_1F_2)$] and wollastonite ($CaO{-}SiO_2$) crystals in a residual $CaO{-}SiO_2$-rich glassy matrix (see Fig. 2). It is available commercially as Cerabone® A-W. A/W glass-ceramic was developed in 1982, by Professors Yamamuro and Kokubo, for use in the repair of the spine [38–41]. The high compressive and bend strengths, 1080 MPa and 215 MPa, respectively [12]; high fracture toughness, 2.0 $MPa^{1/2}$ [12]; high interfacial bond strength to bone [34, 35]; and excellent resistance to degradation of properties when exposed to physiological loading conditions provide confidence in the use of this material to replace surgically removed vertebrae [42]. The mechanical properties of A/W glass-ceramic are far superior to those of Bioglass® 45S5, which has a tensile strength of only 42 MPa, however A/W glass-ceramic exhibits Class B bioactivity [43]. It appears that Peitl and co-workers [44, 45] have overcome the challenge of producing Class A bioactive materials with mechanical properties that are similar to A/W glass-ceramic.

Previous studies of the effect of crystallisation on bioactivity has yielded varying results. Li *et al.* [46] showed that a bioactive glass can be transformed into an inert glass-ceramic, with HCA formation occurring *in vitro* only if the glass-ceramic contained more than 90% of a residual glassy phase. However Peitl *et al.* [45] showed that crystallisation of Bioglass® 45S5 did not inhibit HCA formation, even when fully crystallised, although the onset time for HCA layer formation did decrease with increased crystallinity. The recent Peitl *et al.* study [44] is one of few papers which studies the effect of crystallisation on the rate of HCA layer formation for a series of glass-ceramic compositions.

Melt derived glasses of various compositions were produced without and with different amounts of P_2O_5 [44]. The nominal compositions of SS, 1.07N2C3S, SSP4 and SSP6 are shown in Table III. These glasses were crystallised to varying extents by a two-step heat treatment.

Table IV illustrates the combinations of time and temperature employed for nucleation and subsequent growth of crystals in the glasses.

The crystal phases in all the glass-ceramics produced, determined by X-ray diffraction (XRD) analysis, was $Na_2Ca_2Si_3O_9$ ($1Na_2O{\cdot}2CaO{\cdot}3SiO_2$), in accordance with the crystal phase found in P_2O_5-free glass-ceramics by Moir and Glasser [47]. The presence of

TABLE III Glass compositions studied (wt%) by Peitl *et al.* [44]

Component	SiO_2	Na_2O	CaO	P_2O_5
1.07N2C3S	50.3	18.5	31.3	0.0
SS	50.5	24.8	24.8	0.0
SSP4	48.5	23.8	23.8	4.0
SSP6	47.5	23.2	23.2	6.0
Bioglass® 45S5	45	24.5	24.5	6.0

TABLE IV Thermal treatment ranges used by Peitl *et al.* to produce different crystal volumes [44]

	Nucleation		Growth		
Composition	Temp. (°C)	Time (min)	Temp. (°C)	Time (min)	Volume (%) crystallised
1.07N2C3S	600	960	690	60	100
SS	520–590	3–180	620–640	6–22	10–100
SSP4	540–590	30–6000	650–700	5–80	5–100
SSP6	540–590	60–9000	650–700	10-70	10–100

up to 6% P_2O_5 did not result in the precipitation of another crystal phase in SSP4 and SSP6. This is in contrast to the discovery, by Li *et al.*, of an apatite-like phase, $Ca_{10}(PO_4)_6$, in a partially crystallised glass-ceramic containing $48SiO_2$, $9.5P_2O_5$, $20Na_2O$ and 22.5CaO (wt%). However the Li *et al.* study utilised higher temperatures and shorter times in their heat treatment regime. Peitl *et al.* subjected samples of fully crystallised SSP4 and SSP6 glass-ceramics to a third stage of heat treatment, at 820°C for up to 37 h. XRD and Fourier transform infra-red (FTIR) analyses confirmed the presence of an apatite phase only after this third treatment step, indicating that, in compositions with up to 6% P_2O_5, the P ions remain in solid solution for ordinary thermal treatments. Li *et al.* [46] found that with 9% P_2O_5 an apatite-like phase was precipitated during ordinary crystallisation.

Peitl *et al.* performed *in vitro* bioactivity tests on the glasses and glass-ceramics, under static conditions, by soaking them in simulated body fluid (SBF) for up to 96 h at 37°C. SBF, developed by Kokubo *et al.* [48], is a Tris-hydroxymethlyaminomethane (Tris) buffered solution containing similar ion concentrations to that of human blood plasma, as shown in Table V.

FTIR spectroscopy analysis of the surfaces of the samples and inductively coupled plasma (ICP) spectroscopy of the reacted SBF determined the relative kinetics of the first five reaction stages (described in Table II), those responsible for surface HCA layer formation. All compositions were found to develop an HCA surface layer in SBF. Crystallisation of the phosphorus-free compositions, 1.07N2C3S and SS, had only a slight effect on the onset time for HCA layer formation (from 26 to 32 h). These glasses and glass-ceramics were able to incorporate phosphorus from the SBF to form an HCA layer. Crystallisation of glasses SSP4 and SSP6 increased the onset time of HCA formation from 8 to 24 h. The presence of phosphorus in the glasses and glass-ceramics increased the rate of HCA layer formation but there was no significant difference between the reaction kinetics of the compositions with 4% and 6% P_2O_5. The SSP6 glass, with the closest composition to Bioglass® 45S5 (see Table III), was the fastest to form an HCA layer.

In the Pietl *et al.* study [44], fully crystallised P_2O_5-free glass-ceramics, 1.07N2C3S and SS, were found to be less bioactive, in terms of their *in vitro* reaction kinetics, than the parent glasses but far more bioactive than commercial bioactive ceramics and glass-ceramics, including A/W glass-ceramic, thus indicating that the crystal phase $1Na_2O{\cdot}2CaO{\cdot}3SiO_2$ has a high level of bioactivity. Glass-ceramics containing an apatite-like phase (e.g., commercial synthetic hydroxyapatite ceramic and A/W glass ceramic) are several times less reactive than materials with phosphorus in solid solution, such as Bioglass® 45S5 [44]. Comparing the ionic release of partially crystallised SSP4 and Bioglass® 45S5 Peitl *et al.* determined that they exhibited comparable reaction kinetics, slightly in favour of 45S5. This important finding indicates that the glass-ceramic SSP4 is a "unique crystalline material having comparable bioactivity behaviour to Bioglass® 45S5 but substantially superior mechanical properties" [44]. This suggests that it may be possible to tailor the mechanical properties of bioactive glass-ceramics for use in load-bearing clinical applications. It was also concluded from the *in vitro* study that SSP4 and SSP6 bioactive glasses and glass-ceramics can be classified as Class A bioactive materials, according to Hench's criteria (described above).

TABLE V Ion concentration (mM) in SBF-K9 and in human blood plasma [48]

Ion	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}	SO_4^{2-}
SBF-K9	142.0	5.0	1.5	2.5	147.8	4.2	1.0	0.5
Human plasma	142.0	5.0	1.5	2.5	103.0	27.0	1.0	0.5

Bioactive glass-ceramics have also been produced by a method known as tape casting [49–52]. Tape casting is a promising fabrication technique for bioactive glass-ceramics because complex three-dimensional shapes can be produced, as the final material is built up layer by layer, and the material can be machined in the green state. Clupper *et al.* have shown that tape cast and sintered (TCS) Bioglass® 45S5 can exhibit a well established HA surface layer after *in vitro* dissolution in SBF [49] and Tris-buffer [50]. Four sintering schedules were employed: 800, 900 and 1000°C for 3 h and 1000°C for 6 h. The major crystalline component of the TCS Bioglass® samples was determined by XRD analysis to be $Na_2Ca_2Si_3O_9$, agreeing with the findings of Peitl *et al.* [44] and Moir and Glasser [47]. The samples sintered at 1000°C, while requiring a longer period of time for the initial nucleation and growth of surface HA in solution, were found to exhibit thicker HA layers between 24 h and 8 weeks than the samples sintered at lower temperatures [49, 50]. The sample densification increased with sintering temperature, thereby reducing the surface area available for reaction. Previously it had been shown that 60–100% crystalline Bioglass® 45S5 required 40 h to form a well established HA layer in SBF [53]. The Clupper *et al.* study reports 20–24 h for comparable HA formation on the TCS bioactive glass-ceramic samples sintered at 1000°C [49].

The biaxial flexural strength of the above samples was measured using a piston on ring apparatus [50]. The flexural strength was found to increase with sintering temperature, as porosity decreased, and that of the samples sintered at 1000°C for 3 h was found to increase from 87 to 120 MPa after 14 days of immersion in Tris buffer due to the resultant HA layer formation [50]. Clupper *et al.* also showed that laminating tape cast Bioglass® 45S5 with stainless steel 316L improved the work of fracture of biaxial flexure samples by 109% [51]. After crack propagation through the Bioglass® tensile side the crack was deflected along the Bioglass®-steel interface and re-nucleated on the Bioglass® compressive side. Some plastic deformation of the steel layer was observed. Lamination resulted in no significant reduction in flexural strength [51], indeed the strength is expected to increase with *in vitro* HA layer formation [50]. Equivalent bioactive responses were demonstrated between monolithic

and metal-laminated tape cast Bioglass® [52], despite concerns that hot-pressing might result in the diffusion of polyvalent metal ions into the Bioglass® layers and/or loss of two glass constituents, Na^+ and P^{5+}, with vapour pressure. Both mechanisms could potentially inhibit HA formation *in vitro*. Further examination of the processing and design variables is likely to improve the mechanical properties of this bioactive composite. These materials [49–52] have potential suitability for use in tissue engineering studies where cells are seeded onto materials which have been pre-reacted *in vitro* to form HA layers and also for load-bearing clinical applications.

4. Bioactive resorbable scaffolds

4.1. The ideal scaffold

There are many criteria for an ideal tissue engineering scaffold. Firstly the material used should be biocompatible, i.e., not cytotoxic. The material should bond with the host tissue without the formation of non-adherent scar tissue, i.e., be class A bioactive, and should exhibit a surface texture which promotes this bond, through enhanced cell adhesion and adsorption of biological metabolites. The dissolution products of the material should stimulate genes in the regenerating tissue to promote efficient cell differentiation and proliferation. The structure of the scaffold should act as a template for three-dimensional tissue growth and consist of a highly interconnected porous network. Interconnections greater than 50 μm in diameter [54] and pores larger than 100 μm in diameter [55] should allow for cell penetration, tissue ingrowth, vascularisation and nutrient delivery to the regenerating tissue and also assure mineralised bone formation. The scaffold should resorb at a similar rate as tissue is replaced, while producing degradation products which are non-toxic and can be easily excreted by the body, for example through the respiratory or urinary systems. The mechanical properties of the scaffold should be sufficient to enable tissue regeneration in load-bearing sites. The processing route employed to produce the scaffold should allow for the fabrication of irregular shapes, to match those of defect sites, and should possess the potential to produce scaffolds to the required International Standards Organisation (ISO) or Food and Drug Administration (FDA) standards.

Bioactive gel-glasses fulfil some of the above criteria for tissue engineering scaffolds. Certain compositions are class A bioactive materials and they exhibit an inherent mesoporosity, which enhances interfacial bonding. They exhibit bioresorbability, which can be tailored by controlling the pore texture [31, 32]. Their degradation products are non-toxic and their ionic dissolution products are the same as for melt derived bioactive glasses. The dissolution products of Bioglass® 45S5 have been found to stimulate genes which control the cell cycle leading to differentiation and proliferation of bone cells, as described above [16]. The limiting factor in the use of bioactive gel-glasses as tissue engineering scaffolds is the inherent brittleness of glass. Macroporous glasses, with thin interconnecting walls, are likely to be very weak and unsuitable for load-bearing applications. However, the mechanical properties of sol-gel derived glasses could be optimised by prolonged ageing (coarsening of the gel network). The effect of densification of gel-glass foams on their mechanical properties and *in vitro* dissolution kinetics is currently under investigation at Imperial College, London.

4.2. Macroporous sol-gel derived glasses

Sepulveda and co-workers developed a foaming process to produce macroporous gel-glasses [33]. This process was based upon the gel-casting technique previously developed by Sepulveda to produce macroporous ceramics [56]. This involved the direct foaming of a colloidal suspension of ceramic powders followed by gel casting in which *in situ* polymerisation of organic monomers creates a crosslinked polymeric network (gel). This concept was applied to sol-gel processing by foaming small quantities of the sol using vigorous mechanical agitation, a surfactant and a gelling agent. Surfactants act to lower the surface tension of gas-liquid interfaces, thus making foam films thermodynamically stable. The gelling agent (5 vol% HF) catalysed the polycondensation reaction such that gelling of the foamed sol could be controlled to occur directly after casting. The glass foams produced exhibited a hierarchical structure with interconnected macropores (10–500 μm) and a mesoporous framework (pores of 2–50 nm), thus fulfilling the pore size criterion for tissue engineering scaffolds. The processing route employed allows for the net-shape casting of irregular shapes, both large and small, controlled by mould selection.

Sepulveda and co-workers chose to produce gel-glass foams of compositions from unary, binary and ternary silica-based systems, namely pure SiO_2, 70% SiO_2 30% CaO (S70C30) and 60% SiO_2 36% CaO 4% P_2O_5 (58S). The composition 58S has been studied extensively and found to exhibit high bioactivity *in vitro* and *in vivo* [57–59]. Saravanapavan [60] found the S70C30 composition to have the highest level of bioactivity among a selection of gel-glass compositions in the binary $CaO{-}SiO_2$ system. Work is currently under way by the present authors to find the optimum bioactive composition in this system, in term of the enthalpies of formation and textural control.

5. Molecularly tailored bioactive scaffolds

In order to produce cell-seeded constructs researchers have tried to incorporate into biomaterials bioactive peptides, such as growth factors and proteins. Bioactive peptides can promote cell-surface recognition and also promote or control many aspects of cell physiology, such as adhesion, spreading, activation, migration, proliferation and differentiation [61]. They can also be incorporated into biomaterials to be released in a controlled manner, producing a beneficial effect on tissue formation [62]. While polymeric systems for drug and protein delivery are numerous, there are presently no biomaterials which exhibit simultaneously controlled biomolecule release and bioactive behaviour

[63]. There exists a goal to synthesise materials which release proteins in a controlled way and at the same time serve as a support for tissue ingrowth and direct organogenesis.

Incorporation of bioactive peptides into three-dimensional scaffolds presents a challenge due to the fragile nature and chemical and geometric complexity (conformation) of these macromolecules [64]. The way in which the biomolecules interact with the surface of a material can alter, or hinder, their biological functionality. In order to prevent this peptides must adsorb specifically and maintain conformation. Chemical groups, such as amine and mercaptan groups, are known to control the ability of surfaces to interact with proteins [63, 65]. In addition, these groups can allow protein-surface interactions to occur such that the active domains of the protein can be oriented outwards where they can be maximally effective in triggering biospecific processes [63].

Lenza and co-workers [66, 67] have succeeded in modifying the surface of bioactive sol-gel derived macroporous scaffolds with amine and mercaptan groups. The chemical groups were adsorbed onto the surfaces of S70C30 and 58S gel-glass foams. The protein laminin, a component of the extracellular matrix, is known to promote cell adhesion, proliferation and differentiation [68]. Laminin was adsorbed onto the surfaces of modified and unmodified scaffolds and its release into phosphate-buffered solution (PBS) was monitored for up to 30 days [67]. The secondary structure of laminin released into solution after 2 weeks of immersion was found to differ only slightly from that of laminin in PBS, indicating that the protein conformation was preserved to a considerable extent after 2 weeks of adsorption onto the modified and unmodified foams [67]. Rapid laminin release was observed for the first 24 h and as immersion time increased further, the process became slower. Less than 10% of the protein was released from the bioactive foams within the first 24 h. This is in contrast with the results of other protein release experiments in the literature, where other protein carriers, such as calcium phosphate coatings, release the majority of proteins within the first 48 h of immersion [69]. After 30 days, in the Lenza *et al.* study, less than 40% of the protein had been released, suggesting that the bioactive sol-gel derived foams have the potential to act as scaffolds for tissue engineering with a controlled release of proteins that can induce tissue formation or regeneration. For both the S70C30 and 58S foam compositions the amount of laminin released after 30 days was larger for non-modified foams, indicating that the incorporation of the amine and mercaptan groups assists the control of protein release from the foams. The amount of laminin released from the binary S70C30 foam modified with mercaptan was much lower than that released from the other binary foams, whereas the laminin release from the ternary 58S foam modified with amine was a little lower than for the other ternary foams [67]. The effects of the adsorption of amine and mercaptan groups and laminin on the bioactivity of the foams was monitored by immersing them in SBF for up to 7 days. The reaction kinetics of these foams, observed by measuring the concentration of ionic dissolution products in the reacted SBF by ICP analysis, were similar to those previously observed for laminin release [67], indicating that the laminin release is driven by the dissolution rate of the material. This suggests that the texture of the material is a major variable in the protein release kinetics for bioactive gel-glass foams, as it is for dissolution kinetics. The texture of these foams can be easily controlled during processing, thus enabling optimisation of dissolution and protein release kinetics.

Lenza and co-workers also successfully incorporated amine and mercaptan groups into the bulk composition of pure silica gel-glasses [65]. Albumin is the major protein of human plasma and is known to enhance the biocompatibility of implants. Bovine serum albumin (BSA), which is analogous to human serum albumin, was also added to the bulk composition to facilitate the investigation of these glasses as matrices for controlled release of proteins [70]. The monolith synthesis utilised lower temperatures than those typical for sol-gel synthesis. Drying was performed at 37°C to prevent denaturation of the protein. The protein was found to act as a nucleus for silica network condensation and as a structural template during the gelation of the sol. The pores conformed to the size of the protein, whereas the pores of the protein-free control glasses collapsed upon the evaporation of pore liquid [70]. The average pore size of the BSA-containing glasses was observed to increase after immersion of the glasses in PBS. This was attributed to "silica sol-gel ageing by dissolution-precipitation reactions in aqueous salt solution" [71]. Initially, BSA was released into the PBS in a controlled manner, with a sharp increase after 2 weeks of immersion, a consequence of the increasing pore size and pore volume of the glasses. This suggests that the protein release occurred by two mechanisms: dissolution of the silica network (as for the protein-adsorbed foams, above) and diffusion through the porous network. The increasing pore size would have facilitated the diffusion process, which is likely to have been the rate determining mechanism. The glasses modified with amine groups were found to release less BSA than the unmodified glasses, after 30 days, while the glasses modified with mercaptan groups released more BSA [70]. This suggests that amine groups are more effective at controlling the release of BSA from these glasses and is possibly because the amino acid sequence of BSA more closely matches the structure of amine modified glasses than mercaptan modified glasses. After 30 days of immersion in PBS the amount of BSA released from the glasses was less than 20% of the total protein incorporated, indicating that these chemically modified materials have a high potential to be used as devices for controlled drug and protein delivery and for sustained release [70]. The release rate can be tailored by controlling the matrix composition and texture.

6. Bioactive composite scaffolds

The complex requirements of the ideal scaffold for tissue engineering may only be completely fulfilled

by a material with complex properties. The field of bioceramic composites is a broad one, reviewed elsewhere [72]. Each new biomaterial is designed or developed to fulfil a specific clinical need. The first bioceramic composites were designed to match the natural components of bone by replacing natural hydroxyapatite with a synthetic particulate HA and collagen with polyethylene (PE) [73]. The resultant material was developed to optimise mechanical properties and bioactivity, by varying the volume fraction of HA [74]. Under the trade name HAPEX™ the HA-PE composite has been successfully used to partially or totally replace the bones of the middle ear to correct conductive hearing loss [72]. Composites containing Bioglass® 45S5 and a bioactive glass ceramic as the second phase, with or without ductile metal lamination, as discussed above, can be classified as bioactive glass composites. They can provide long term mechanical support or stability to a defective bony site, like the HAPEX™ material, but they have the advantage of enhanced osteoproductive properties which accelerate bone bonding and facilitate the repair of soft tissue structures.

To allow for bone regeneration and augmentation, a resorbable composite is desirable. Either the matrix or both the matrix and the second phase can be resorbable materials. Resorbable matrices can be used as transient carrier systems, allowing bioactive particles to be delivered to a specific site within the body. Composites containing hydroxyapatite with collagen received much attention as copies of the natural bone composition [72]. Gelatine was investigated as a matrix for use with HA [75] as it resorbs quickly and reduces the amount of time it takes for the HA particles to become active. However the system was found to lose mechanical strength rapidly allowing particles to migrate.

Recent work by Roether *et al.* [76] has shown that resorbable polymer foams, coated with Bioglass® 45S5, are bioactive. Poly(DL-lactic acid) (PDLLA) has been shown to degrade *in vitro* without generation of any crystalline remnants [76]. PDLLA has been shown to resorb completely *in vivo* with no inflammation of surrounding tissue [77]. PDLLA foams were fabricated following a thermally induced phase separation (freeze-drying) and coated with a slurry of Bioglass® particles, via two methods: slurry-dipping and electrophoretic deposition (EPD) [76]. Scanning electron microscopy (SEM) analysis revealed the morphology of the prepared composite foams to contain two distinct pore sizes; macropores of $\geq$100 μm and 20–30 μm average diameter which formed an interconnected network [76]. The tubular macropores ($\geq$100 μm) were highly oriented due to the unidirectional cooling process employed. Macropores of diameter $\geq$100 μm are a requirement for successful tissue ingrowth on tissue engineering scaffolds. Slurry-dipping resulted in a thin, even film of Bioglass® covering the surface of the foam, homogeneous inside the pores and on the outer surface. No peeling-off or macro-delamination of the coating was observed. SEM analysis showed that the Bioglass® coating had a porous texture, due to the narrow size distribution of the glass particles used [76]. The microstructure of the Bioglass® was highly reproducible by this technique. EPD deposited a thick layer of Bioglass® particles on the outer surface of the foam and efficiently filled both size distributions of macropores. It was concluded [76] that for the intended tissue engineering application of the prepared foam composites slurry-dipping offered the more suitable coating method, as the coating thickness could be more easily controlled and cell proliferation requires the scaffold to possess sufficient pore volume and pores of given, controlled size.

Bioglass®-coated PDLLA foams were found to form an HA layer when immersed in SBF, whereas the uncoated foam did not produce HA after 28 days of immersion [76]. After 7 days of immersion the presence of an HA layer on the Bioglass®-coated foams was confirmed by X-ray diffraction (XRD) analysis. Small crystals deposited after the first week developed into a continuous HA layer formed by coalescence of large crystals after 3 weeks [76]. No quantitative analysis of the amount of HA formed in relation to immersion time in SBF was reported as the foam samples used in the study differed in size and morphology.

Recently, Day *et al.* have investigated the biocompatability of a polyglycolic acid (PGA) mesh, coated with Bioglass® 45S5, placed subcutaneously *in vivo* [78]. The sterile knitted sheet (Dexon mesh®) was slurry-dipped to obtain a coating of Bioglass® 45S5 particles. *In vitro* studies were performed to assess the response of fibroblast cells to Bioglass® 45S5. Whereas the vast majority of previous studies on bioresorbable scaffolds containing bioactive phases had focussed on applications in bone, the Day *et al.* study assessed Bioglass® 45S5 for its application in soft tissue engineering. It is the first study to investigate the *in vivo* cellularisation of a PGA-Bioglass® 45S5 composite mesh. It was shown that the Bioglass®-coated meshes stimulated neovascularisation from the surrounding tissue *in vivo* [78], i.e., the number of blood vessels per field view under light microscopy significantly increased with implantation time compared with the uncoated mesh. The *in vitro* studies revealed that low concentrations of Bioglass® coating resulted in an increased secretion of vascular endothelial growth factor (VEGF) from fibroblast cells, compared with uncoated cells [78]. Day suggests this may be the cause of the increased neovascularisation, as VEGF is a highly pro-angeogenic growth factor which acts solely on endothelial cells. Growth factors are polypeptides which transmit signals to modulate cellular activities, such as proliferation, differentiation, migration, adhesion and gene expression. It was found that the coatings used to induce the maximum stimulatory effects in the Day *et al.* study were relatively thin and discontinuous. For example, the *in vitro* studies showed that concentrations of Bioglass® coating $\geq$0.1% inhibited cell proliferation and VEGF release, compared with that of uncoated cells [78]. Day suggests that this may be due to a shift towards a more differentiated cell phenotype.

The mesh used in the Day *et al.* study had spaces between the fibres large enough to accommodate well-vascularised developing tissue. This is important because as the PGA decomposes (60–90 days) the tissue

will have to maintain its own structural integrity. The flexurability of PGA makes the construction of shapes other than flat sheets possible, such as tubular scaffolds. The PGA-Bioglass® 45S5 composite mesh exhibited good biocompatability *in vivo*, indicating that it may have potential use in the development of soft-tissue organ constructs.

7. Future directions

This paper has reviewed some of the most significant findings reported in the last few years regarding third generation biomaterials. It was discussed how the concepts of bioactivity and resorbability have been combined to produce biomaterials capable of stimulating proliferation of cells while possessing the ability to resorb and allow for the regeneration and augmentation of living tissue.

The ionic dissolution products of Bioglass® 45S5 have been shown to control the genes that regulate cell cycle induction and progression, thereby stimulating the proliferation of osteoblast cells [16–20, 22]. Bioglass® 45S5 has been used to coat the surface of resorbable macroporous PDLLA foams, thus imparting a bioactive response to the composite, which formed HA on its surface upon immersion in SBF [76]. Bioglass® 45S5 was also shown to enhance the proliferation of, and release of vascular endothelial growth factor from, fibroblast cells coated with low concentrations of Bioglass® 45S5 [78]. A Bioglass®-coated polyglycolic acid (PGA) mesh stimulated neovascularisation when implanted subcutaneously into rats [78]. The resorbable PGA fibres can be assembled into different shaped constructs and when coated with Bioglass® 45S5 has the potential to be used as a scaffold for the engineering of soft tissues.

Sintering of bioactive glasses, including tape cast Bioglass® 45S5, has resulted in the crystallisation of a highly bioactive phase, $Na_2Ca_2Si_3O_9$ [44, 49–52]. Lamination of tape cast Bioglass® 45S5 with stainless steel 316L resulted in the toughening of the glass [51]. These findings have positive implications for the use of Class A bioactive glasses and glass-ceramics in load-bearing clinical applications.

Glasses fabricated via the sol-gel process possess an inherent nanometre scale porosity which enhances their bioactivity in comparison to melt derived bioactive glasses. The processing route allows for the production of resorbable macroporous foams, ideal for tissue engineering, which can be cast into complex three-dimensional shapes [33]. The effects of densification on the mechanical properties of these foams are currently being studied. It was shown that bioactive peptides, which can promote many aspects of cell physiology during tissue engineering, can be successfully adsorbed onto the surface of bioactive sol-gel glass foams [67]. The successful incorporation of proteins onto the surface of gel-glass foams and into the bulk composition of pure silica gel-glasses, as well as the controlled release rates of these proteins into solution over time periods of up to 30 days, suggest that these materials would be ideal for use as carrier systems for tissue engineering and drug delivery.

These recent findings indicate that a biologically based method for the repair and regeneration of tissues shows considerable promise. Materials can stimulate the body's own regenerative capacity and biologically active molecules are being incorporated into these materials to improve this response. Future implications of these findings are revolutionary. Gene-activating biomaterials have the potential to be tailored for specific patients and disease states. Tissue engineered constructs could be produced based on a patient's own cells to reduce the risk of rejection. It may also be possible to develop a preventative treatment to maintain the health of the body's tissues with age, using bioactive stimuli to activate genes. With the increased life expectancy of people in developed countries, the implication of this kind of treatment is body parts which retain their functionality for the patient's whole life.

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References

1. R. LANGER and J. P. VACANTI, *Science* **260** (1993) 920.
2. F. BERTHIAUME and M. L. YARMUSH, in "The Biomedical Engineering Handbook," edited by J. D. Bronzino (CRC Press LLC, Boca Raton, Florida, 2000).
3. L. L. HENCH and J. M. POLAK, *Science* **295** (2002) 1014.
4. L. L. HENCH, *ibid.* **208** (1980) 826.
5. *Idem.*, *J. Amer. Ceram. Soc.* **81** (1998) 1705.
6. L. L. HENCH and J. WILSON (eds.), "Clinical Performance of Skeletal Prostheses" (Chapman and Hall, London, 1996).
7. B. M. WROBLEWSKI, P. A. FLEMING and P. D. SINEY, *J. Bone Jt. Surg. Br.* **81** (1999) 427.
8. F. J. SCHOEN, R. J. LEVY and H. R. PIEHLER, *J. Soc. Cardiovasc. Pathol.* **1** (1992) 29.
9. L. L. HENCH, R. J. SPLINTER, W. C. ALLEN and T. K. GREENLEE, JR., *J. Biomed. Mater. Res.* **2** (1971) 117.
10. C. A. BECKHAM, T. K. GREENLEE, JR. and A. R. CREBO, *Calc. Tiss. Res.* **8** (1971) 165.
11. T. K. GREENLEE, JR., C. A. BECKHAM, A. R. CREBO and J. C. MALMBORG, *J. Biomed. Mater. Res.* **6** (1972) 235.
12. L. L. HENCH, *J. Amer. Ceram. Soc.* **74** (1991) 1487.
13. L. L. HENCH and J. WILSON (eds.), "Introduction to Bioceramics" (Singapore World Scientific, Singapore, 1993).
14. J. WILSON, G. H. PIGOTT, F. J. SCHOEN and L. L. HENCH, *J. Biomed. Mater. Res.* **15** (1981) 805.
15. J. WILSON and D. NOLLETTI, in "Handbook of Bioactive Ceramics," edited by T. Yamamuro, L. L. Hench and J. Wilson (CRC Press, Boca Raton, Florida, 1990) Vol. 1, p. 283.
16. I. D. XYNOS, M. V. J. HUKKANEN, J. J. BATTEN, I. D. BUTTERY, L. L. HENCH and J. M. POLAK, *Calcif. Tissue Int.* **67** (2000) 321.
17. L. L. HENCH, I. D. XYNOS, L .D. BUTTERY and J. M. POLAK, *J. Mater. Res. Innovations* **3** (2001) 313.
18. I. D. XYNOS, A. J. EDGAR, L. D. BUTTERY, L. L. HENCH and J. M. POLAK, *Biochem. and Biophys. Res. Comm.* **276** (2000) 461.
19. *Idem.*, *J. Biomed. Mater. Res.* (2001).
20. L. L. HENCH, *Key Engin. Mater.* **192–195** (2001) 575.
21. D. HUGHES, D. M. SALKER and R. SIMPSON, *J. Bone Miner. Res.* **9** (1994) 39.
22. L. L. HENCH, I. D. XYNOS, A. J. EDGAR, L. D. K. BUTTERY and J. M. POLAK, in Proc. Int. Congr. Glass Volume 1, Invited Papers (Edinburgh. Scotland, 1–6 July 2000) p. 226.
23. L. L. HENCH and J. K. WEST, *Chem. Rev.* **90** (1990) 33.

24. K. ISHIZAKI, S. KOMARNENI and M. NANKO, "Porous Materials. Process Technology and Applications" (Kluwer Academic, Dordrecht, 1998) p. 67.
25. J. LIVAGE and C. SANCHEZ, *J. Non-Cryst. Solids* **145** (1992) 11.
26. R. LI, A. E. CLARK and L. L. HENCH, in "Chemical Processing of Advanced Materials," edited by L. L. Hench and J. K. West (Wiley, New York, 1992) p. 627.
27. M. M. PEREIRA and L. L. HENCH, *J. Sol-Gel Sci. Technol.* **7** (1996) 59.
28. L. L. HENCH, D. L. WHEELER and D. C. GREENSPAN, *ibid.* **13** (1998) 245.
29. M. M. PEREIRA, A. E. CLARK and L. L. HENCH, *J. Biomed. Mater. Res.* **28** (1994) 693.
30. L. L. ELSBURG, K. D. LOBEL and L. L. HENCH, unpublished work.
31. D. C. GREENSPAN, J. P. ZHONG, Z. F. CHEN and G. P. LATORRE, *Bioceramics* **10** (1997) 391.
32. M. M. PEREIRA, A. E. CLARK and L. L. HENCH, *J. Amer. Ceram. Soc.* **78** (1995) 2463.
33. P. SEPULVEDA, J. R. JONES and L. L. HENCH, *J. Biomed. Mater. Res.* **59** (2002) 340.
34. L. L. HENCH and J. K. WEST, *Life Chem. Rep.* **13** (1996) 187.
35. J. LIVAGE, *Curr. Opin. Solid State Mater. Sci.* **2** (1997) 132.
36. A. AKSAY and S. WEINER, *ibid.* **3** (1998) 219.
37. T. NAKAMURA, T. YAMAMURO, S. HIGASHI, T. KOKUBO and S. ITOO, *J. Biomed. Mater. Res.* **19** (1985) 685.
38. T. KOKUBO, S. ITO, S. SAKKA and T. YAMAMURO, *Mater. Sci.* **21** (1986) 536.
39. T. KITSUGI, T. YAMAMURO and T. KOKUBO, *J. Bone Jt. Surg.* **71A** (1989) 264.
40. S. YOSHII, Y. KAKUTANI, T. YAMAMURO, T. NAKAMURA, T. KISUGI, M. OKA, T. KOKUBO and M. TAKAGI, *J. Biomed. Mater. Res.* **22A** (1988) 327.
41. T. YAMAMURO, J. SHIKATA, Y. KAKUTANI, S. YOSHII, T. KITSUGI and K. ONO, in "Bioceramics: Materials Characteristics Versus In Vivo Behavior," edited by P. Ducheyne and J. Lemons (Annals of New York Academy of Science, New York, 1988) Vol. 523, p. 107.
42. T. KOKUBO, "A/W Glass-Ceramic: Processing and Properties"; see Ref. 13, Ch. 6.
43. J. R. JONES and L. L. HENCH, *Mater. Sci. Technol.* **17** (2001) 891.
44. O. PEITL, E. D. ZANOTTO and L. L. HENCH, *J. Non-Cryst. Solids* **292** (2001) 115.
45. O. PEITL, G. LATORRE and L. L. HENCH, *J. Biomed. Mater. Res.* **30** (1996) 509.
46. P. LI, F. ZHANG and T. KOKUBO, *J. Mater. Sci. Mater. Med.* **3** (1992) 452.
47. G. K. MOIR and F. P. GLASSER, *Phys. Chem. Glasses* **15** (1974) 6.
48. T. KOKUBO, H. KUSHITANI and S. SAKKA, *J. Biomed. Mater. Res.* **24** (1990) 721.
49. D. C. CLUPPER, J. J. MECHOLSKY JR., G. P. LATORRE and D. C. GREENSPAN, *Biomaterials* **23** (2002) 2599.
50. *Idem*., *J. Biomed. Mater. Res.* **57** (2001) 532.
51. D. C. CLUPPER and J. J. MECHOLSKY, JR., *J. Mater. Sci. Lett.* **20** (2001) 1885.
52. D. C. CLUPPER, J. J. MECHOLSKY, JR., G. P. LATORRE and D. C. GREENSPAN, *ibid.* **20** (2001) 959.
53. O. P. FILHO, G. P. LATORRE and L. L. HENCH, *J. Biomed. Mater. Res.* **30** (1996) 509.
54. J. X. LU, B. FLAUTRE, K. ANSELME, P. HARDOUIN, A. GALLUR, M. DESCAMPS and B. THIERRY, *J. Mater. Sci. Mater. Med.* **10** (1999) 111.
55. T. M. FREYMAN, I. V. YANNAS and L. J. GIBSON, *Prog. Mater. Sci.* **46** (2001) 273.
56. P. SEPULVEDA and J. G. P. BINNER, *J. Eur. Ceram. Soc.* **19** (1999) 2059.
57. R. LI, A. E. CLARK and L. L. HENCH, *Chem. Proc. Adv. Mater.* **56** (1992) 627.
58. R. COOK, E. FIELDER, T. WATSON, P. ROBINSON and L. L. HENCH, *Key Eng. Mater.* **192–195** (2000) 625.
59. L. L. HENCH, *Curr. Opin. Solid State Mater. Sci.* **2** (1997) 604.
60. P. SARAVANAPAVAN and L. L. HENCH, *J. Biomed. Mater. Res.* **54** (2001) 608.
61. P. D. DRUMHELLER and J. A. HUBBELL, in "The Biomedical Engineering Handbook," edited by J. D. Bronzino (CRC Press LLC, Boca Raton, Florida, 2000) Vol. 2.
62. M. H. SHERIDAN, L. D. SHEA, M. C. PETERS and D. J. MOONEY, *J. Control. Release* **64** (2000) 91.
63. K. E. HEALY, *Curr. Opin. Solid St. M.* **4** (1999) 381.
64. B. D. RATNER and H. SHI, *ibid.* **4** (1999) 395.
65. H. S. MANSUR, W. L. VASCONCELOS, R. F. S. LENZA, R. L. ORÉFICE, E. F. REIS and Z. P. LOBATO, *J. Non-Cryst. Solids* **273** (2000) 109.
66. R. F. S. LENZA, J. R. JONES, W. L. VASCONCELOS and L. L. HENCH, *J. Mater. Sci. Mater. Med.* **13** (2002) 837.
67. *Idem.*, "*In Vitro* Release Kinetics of Proteins from Bioactive Foams," in press.
68. S. B. NICHOLL, S. RADIN, E. M. SANTOS, R. S. TUAN and P. DUCHEYNE, *Biomaterials* **18** (1997) 853.
69. F. LEKMINE, S. LAUSSON, E. PIDOUX, N. SEGOND, B. ROOS, F. TRELHOU-LAHILLE and N. JEANNE, *Mol. Cell. Endocr.* **157** (1999) 181.
70. R. F. S. LENZA, J. R. JONES, W. L. VASCONCELOS and L. L. HENCH, "Controlled Protein Release from Silica-Based Sol-Gel Glasses," in press.
71. C. J. BRINKER and G. W. SCHERER, "Sol-Gel Science: The Physics and Chemistry of Sol-Gel Processing" (Academic Press, San Diego, 1990).
72. I. THOMPSON and L. L. HENCH, in "Comprehensive Composite Materials," edited by A. Kelly and C. Zweben (Elsevier Science, 2000) Vol. 6.39, p. 727.
73. W. BONFIELD, M. D. GRYNPAS, A E. TULLY, J. BOWMAN and J. ABRAM, *Biomaterials* **2** (1981) 185.
74. W. BONFIELD, R. Z. LEGEROS and J. P. LEGEROS (eds.), in "Composite Biomaterials: Present and Future" (World Scientific, Singapore, 1998) Vol. 11, p. 37.
75. K. S. TENHUISEN and P. W. BROWN, *J. Biomed. Mater. Res.* **28** (1994) 27.
76. J. A. ROETHER, A. R. BOCCACCINI, L. L. HENCH, V. MAQUET, S. GAUTIER and R. JÉRÔME, *Biomaterials* **23** (2002) 3871.
77. W. HEIDMAN, S. JESHKEIT, K. RUFFIEUX, J. H. FISCHER, M. WAGNER, G. KRÜGER, E. WINTERMANTEL and K. GERLACH, *ibid.* **22** (2001) 2371.
78. R. M. DAY, A. R. BOCCACCINI, S. SUREY, J. A. ROETHER, A. FORBES, L. L. HENCH and S. M. GABE, *ibid.* in press, 2003.