Bioactive glasses beyond bone and teeth: Emerging applications in contact with soft tissues

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Abstract

The applications of bioactive glasses (BGs) have to a great extent been related to the replacement, regeneration and repair of hard tissues, such as bone and teeth, and there is an extensive bibliography documenting the role of BGs as bone replacement materials and in bone tissue engineering applications. Interestingly, many of the biochemical reactions arising from the contact of BGs with bodily fluids, in particular the local increase in concentration of various ions at the glass–tissue interface, are also relevant to mechanisms involved in soft tissue regeneration. An increasing number of studies report on the application of BGs in contact with soft tissues, aiming at exploiting the well-known bioactive properties of BGs in soft tissue regeneration and wound healing. This review focuses on research, sometimes involving preliminary in vitro studies but also in vivo evidence, that demonstrates the suitability of BGs in contact with tissues outside the skeletal system, which includes studies investigating vascularization, wound healing and cardiac, lung, nerve, gastrointestinal, urinary tract and laryngeal tissue repair using BGs in various forms of particulates, fibers and nanoparticles with and without polymer components. Potentially active mechanisms of interaction of BGs and soft tissues based on the surface bioreactivity of BGs and on biomechanical stimuli affecting the soft tissue–BG collagenous bonding are discussed based on results in the literature.

1. Introduction and historical background

The use of bioactive glasses (BGs) and glass ceramics as suitable biomaterials for orthopedic and dental applications, as well as in bone tissue engineering, has been well documented and comprehensive review articles have been published recently [1–6]. The important characteristics of BGs as bioactive systems suitable for bone and tooth repair and regeneration, such as a high bioactivity, osteoconduction and osteostimulation, have been well established by numerous investigators in the last 40 years since the seminal report of the discovery of bioactive glass by Hench et al. in 1971 [7,8]. Classical applications of BGs involve bioactive coatings on orthopedic implants [9,10], bone filling materials, dental applications and small bone implants [4,11,12]. The most widely investigated BG composition is the bioactive silicate “45S5 Bioglass”, which has a composition (in wt.%) of 45% SiO₂, 24.5% each of Na₂O and CaO, and 6% P₂O₅ [12], also known as the “grandfather composition” [4]. The “13-93” bioactive silicate glass is also receiving increasing attention [1]. This glass has a higher SiO₂ content (53 wt.%) than 45S5 Bioglass, and the composition includes K₂O and MgO as network modifiers (12 and 5 wt.%, respectively) [13,14]. With respect to clinical applications, the composition “SS3P4” is widely used, with successful outcomes being recorded for the treatment of osteomyelitis [15]. Several other compositions have been developed over the years with the aim of improving the processability and bioactivity of 45S5 Bioglass [1,13,16]. Some typical BG compositions are presented in Table 1.

From the early days of research on BGs, it has been demonstrated that these materials form a direct bond to bone [7,8], and a recent comprehensive review has summarized the knowledge about the interactions between BGs and collagen in the context of both bone/BG and soft tissue bonding [17]. In addition, an important body of research has shown that the ionic products of BG dissolution in body fluid stimulate osteoblast proliferation [18–20] and may enhance angiogenesis under specific in vivo and in vitro conditions [21]. Given the increasing interest in BG applications in medicine and dentistry, a variety of innovative applications are emerging; for example, the use of BGs in the
musculoskeletal system has extended to the successful culture of annulus pulposus cells on PLLA/Bioglass® composite films for intervertebral disk tissue repair [22,23]. Also, BGs in combination with polymers are being considered for interface tissues (hard–soft tissue interfaces) [24–26]. In addition, bioactive glasses have been shown to be effective antibacterial agents against a range of bacteria known to be associated with post-operative infections [27–29] and with implant failure [30,31]. Further, the doping of BGs with trace elements is being increasingly considered to enhance certain characteristics of the glasses: for example, silver has been used to impart increased an enhanced antibacterial effect in BG [32–34]; lithium substituted 45S5 BG has been shown to increase cell proliferation and metabolic activity [35]; copper-doped BGs have been proposed to induce angiogenesis in bone regeneration strategies [36,37]; cobalt-containing glasses with controllable ion release have been developed to mimic hypoxic conditions and enhance angiogenesis [38,39]; and zinc-containing glasses are being considered to increase the proliferation rate of osteoblasts in culture [40]. In addition, Sr-containing BGs are being investigated in the context of strategies to combat osteoporosis [41]. The application of BGs containing Fe is also being considered for applications in cancer treatment by hyperthermia [42,43], while novel applications of BGs are being reported for embolization therapy [44]. Bioactive glasses, especially in their mesoporous form, are also being investigated as drug delivery devices for controlled and longer lasting delivery after implantation [45–50]. Surface modification of BGs has been employed to increase their bioactivity, e.g. by calcium surface enrichment [51], or to increase their biocompatibility by the addition of appropriate biomolecules [52,53] in order to increase interactions between the BG surface and proteins. The use of BGs extend also to cosmetic and dental care applications. For example, BGs in particulate form are added to toothpaste [54–56] and as mineralization agents [57], e.g. in the form of composites containing BG nanoparticles [58–60] or as electrosprun cotton-wool-like templates [61]. In addition, a sodium calcium phosphosilicate glass (Vitryxx®) has been extensively tested to investigate skin reaction in humans to high concentrations of the glass in various cosmetic products, ranging from nail to skin care products [62]. Promising applications in ophthalmology and ocular surgery have been also reported [63]. Perhaps not surprisingly, given the inorganic nature and mechanical rigidity and brittleness of BGs, which exhibit physical characteristics closer to “hard” tissues, much less attention has been paid to the role that BGs may have as materials for soft-tissue engineering applications, even though some of the characteristics that make BGs ideal for bone or dental applications are also key to soft tissue regeneration approaches. The early reports by Wilson et al. [64,65] documented the stable interactions of bioactive glasses and soft tissues. In early pioneering efforts, Wilson et al. [65] carried out numerous in vitro and in vivo experiments to examine the biocompatibility and possible toxicity of Bioglass® in contact with various types of soft tissues. In vitro tests involved seeding diverse cell types from mice, rats, hamsters, chickens and humans onto solid and powdered BG samples with different formulations, including 45S5, 52S4.6 and 45S5F (see Table 1 for their compositions). In vivo testing was carried out by implanting powdered and solid disk samples of various BG compositions subcutaneously, intramuscularly and in the peritoneal cavity of various mammals, including dogs. The in vivo tests demonstrated tissue growth and adhesion around the implants, even when placed in areas where shear stresses applied to the surface of the implants would have produced particulates in micromotion had this not been prevented by strong collagen–BG bonding. Autopsied animals did not show signs of any inflammatory reaction. These early experiments can be considered as initial significant attempts to understand the interaction of bioactive glasses with soft tissues. In fact, the stable soft tissue 45S5 BG implant interface was the basis for the first BG clinical applications of middle ear prostheses and endo-osseous ridge maintenance implants, as reviewed recently in detail by Hench and Greenspan [17]. 45S5 Bioglass® has also been added to high-density polyethylene for applications in more rigid tissues, such as cartilage and connective tissue, which tend to exhibit more difficulties in bonding to prostheses [66]. In other relevant early studies by Gatti et al. [67], S53P4 bioactive glass (see Table 1 for composition) granules of around 300 μm were implanted in the dorsal muscle and under the dorsal skin of rabbits and in defects surgically created in the jaw of sheep to investigate the reactions of soft and hard tissues to this glass. The granules were left in the rabbits for 2 months and in the sheep for 3 months. After excision of the implanted sites and examination of the samples, it was revealed that the nature of the reactions did not depend on the type of tissue the glass was implanted in, the reactions between the BG granules and their surroundings developing in roughly the same manner. Similarly, Meretoja et al. [68] investigated the effect of subcutaneously implanted polycaprolactone/lactide and S53P4 BG composite scaffolds in rats and found that after 4 weeks of implantation there was no inflammatory reaction and only a mild foreign body reaction occurred, while the scaffolds were fully invaded by well-vascularized connective tissue.

Analysis of the literature shows that, for many years, studies focusing on soft tissue/BG interactions were somewhat sporadic; however, great interest in this subject has emerged recently, and an increasing number of studies are being published, which inspired the preparation of this review article to discuss the research related to the potential of BGs to be used in innovative approaches towards regeneration of soft tissues of various types and clinical needs.

The authors intend that this review will serve to fill a gap in the available (and abundant) bioactive glass literature, which mainly focuses on bone and dental applications, by comprehensively covering the applications of bioactive glasses in areas such as angiogenesis, wound healing, hemostasis and regeneration of soft tissues as diverse as nerve, cardiac, lung and intestines (Fig. 1). The review covers mainly silicate bioactive glasses; however,
angiogenesis is applicable to soft tissue engineering approaches. That specific compositions of BGs can have a stimulatory effect on the context of bone tissue engineering [3,73,80–84], the discovery of vascularization in BG-based materials have been carried out by Day et al. [79], and relevant in vitro and in vivo investigations. The angiogenic effect of bioactive glass was first reported in 2004 [45,75,76], there are still many concerns about the duration and efficiency of their delivery to surrounding tissues when implanted, given that these factors are delicate and degrade easily, and controlled release has proved difficult to achieve. Therefore, the alternative of developing (inorganic) materials that promote angiogenesis by their own degradation mechanisms, e.g. delivery of bioactive glass, is very appealing [18,37–39,77,78]. The angiogenic effect of bioactive glass was first reported in 2004 by Day et al. [79], and relevant in vitro and in vivo investigations have been reviewed by Gorustovich et al. [21]. Although most studies of vascularization in BG-based materials have been carried out in the context of bone tissue engineering [3,73,80–84], the discovery that specific compositions of BGs can have a stimulatory effect on angiogenesis is applicable to soft tissue engineering approaches.

Various types of cells, including fibroblasts, myocytes, hepatocytes and neurons, release VEGF under hypoxic conditions. Fibroblasts are known to secrete both VEGF and fibroblast growth factor (FGF), a protein with a powerful mitogenic effect on both fibroblasts and endothelial cells [74]. These growth factors are of importance when investigating angiogenesis [85]. Endothelial cells are crucial for the formation of new blood vessels since they migrate and divide to form tubules that connect to other blood vessels and are affected by the aforementioned growth factors in this process. Whilst growth factors are crucial for neovascularization to occur, there is a need for them to be controlled and localized, and their long-term release must be tailored to avoid malformations in the vascular network, uncontrolled or misplaced tissue growth, and to allow sufficient time for the growth and remodeling of the new vessels [86,87].

To investigate how different concentrations of 45S5 Bioglass® would affect the production of VEGF and basic FGF (bFGF) in human fibroblasts, Day et al. [79] seeded populations of these cells on polymer surfaces coated with different amounts of Bioglass® and incubated them for up to 72 h. In parallel, they prepared poly-glycolic acid (PGA) meshes coated with 45S5 Bioglass® particles at different concentrations which were surgically implanted in subcutaneous pockets in rats (Fig. 2). The cultured fibroblasts were found to secrete VEGF under all conditions. However, as seen in Fig. 2. However, at the higher concentrations investigated (0.1–10% culture plate cover) the secretion of the growth factor was inhibited, indicating a dose-dependent effect, which has also been found by other researchers [21]. In a similar experimental setting, Day [88] examined the effect of Bioglass® on fibroblasts and found that all populations secreted significantly greater amounts of both VEGF and bFGF compared to control cultures without Bioglass®. This increase in growth factor secretion was found to originate from direct stimulation of cells by the Bioglass® particles and not by an increase in the fibroblast population, since the cultures at the concentrations necessary to elicit this phenomenon (0.3125–6.25 mg cm−2) had smaller cell populations compared to the controls. This direct effect of stimulating fibroblasts was confirmed by measuring increased amounts of VEGF mRNA. Conditioned media from these experiments were used to culture endothelial cells, which caused a 61.5% increase in cell population compared to controls, confirming that small amounts of Bioglass® directly stimulate the secretion of angiogenic growth factors by fibroblasts and significantly increase angiogenesis in vitro. The results of the investigation thus provided evidence that BGs accelerate the angiogenic process by stimulating the secretion of relevant growth factors, such as VEGF, through the stimulation of cytokine production in cells [88].

**2. Angiogenesis**

A crucial aspect for the success of scaffolds for tissue engineering and regeneration of vascularized tissues is the need to develop sufficient neovascularization in the affected area in order to ensure suitable transport of nutrients and growth factors into and removal of waste products from the new tissue formation site [73]. Angiogenesis is also crucial for the survival of cells seeded into the scaffold prior to implantation and for the maintenance of any cell populations that may be recruited during tissue formation. The mechanism by which new blood vessels are formed from pre-existing ones, angiogenesis, is regulated by many conditions, including growth factors, e.g. vascular endothelial growth factor (VEGF), extracellular matrix proteins as well as the amount and kind of cell membrane receptors and other signaling molecules available [74]. Although the introduction of growth factors and signaling molecules onto tissue engineering scaffolds is a common approach [45,75,76], there are still many concerns about the duration and efficiency of their delivery to surrounding tissues when implanted, given that these factors are delicate and degrade easily, and controlled release has proved difficult to achieve. Therefore, the alternative of developing (inorganic) materials that promote angiogenesis by their own degradation mechanisms, e.g. delivery of bioactive glass, is very appealing [18,37–39,77,78]. The angiogenic effect of bioactive glass was first reported in 2004 by Day et al. [79], and relevant in vitro and in vivo investigations have been reviewed by Gorustovich et al. [21]. Although most studies of vascularization in BG-based materials have been carried out in the context of bone tissue engineering [3,73,80–84], the discovery that specific compositions of BGs can have a stimulatory effect on angiogenesis is applicable to soft tissue engineering approaches.

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In a more recent study by Gerhardt et al. [89], the angiogenic effect of BGs was investigated by comparing plain poly(D,L-lactide) (PDLLA) and composite PDLLA–BG scaffolds, and it was found that there was a marked increase in the release of VEGF by fibroblasts cultured on the composite scaffolds compared to plain PDLLA films. Similarly, in vivo experiments in a rat model showed that composite scaffolds induced greater vascularization and increased percentages of blood vessel formation, determined by stereological analysis of the vascularized tissues. Another frequently used bioactive silicate glass, S53P4 (see Table 1 for composition), was investigated in a similar model involving human fibroblasts [70]. The cells were cultured in contact with granules of S53P4 BG in three ranges of particle sizes (0.5–1.0, 1.0–2.0 and 2.0–3.15 mm) and in different concentrations (0, 0.01, 0.1 and 1 wt./vol.%). VEGF production was enhanced for particles of 0.5–0.8 and 1.0–2.0 mm at all concentrations, but inhibited by particles of 2.0–3.15 mm. The angiogenic effect of BGs has been also investigated in the chick embryo chorioallantoic membrane (CAM) model by Vargas et al. [90], who found that high concentrations of 45S5 Bioglass® failed to exhibit any pro-angiogenic effects in chick embryos incubated with scaffolds of this material. These cumulative results indicate that an optimum concentration of Bioglass® dissolution products released at a controlled rate is necessary to exploit its angiogenic capabilities.

In a recent study by Handel et al. [91], 45S5 Bioglass® scaffolds prepared by the foam replica method were coated with collagen and bio-functionalized by seeding with human adipose tissue-derived stem cells to be tested in the CAM angiogenesis model, opening the alternative of investigating BGs with ion doping (e.g. Cu, Co), which may be effective in enhancing angiogenesis [36,38,92]. It was found that, compared to plain Bioglass® scaffolds, the biofunctionalized scaffolds showed an increase in vascular tube lengths. However, it is worth noting that, for this particular assay, the release of ionic products from Bioglass® samples was found to have a negligible effect on angiogenesis.

In summary, numerous investigations, some of which have been briefly discussed in this section, have established that BGs can stimulate neovascularization, and it is clear that the ionic products arising from the surface reactions of BGs in contact with the extracellular matrix must be carefully controlled, given that very specific concentrations are necessary for the optimum production or secretion of the necessary growth factors that govern the formation of blood vessels in living tissue. Clearly, the use of BGs as angiogenic agents in tissue engineering approaches is a field in its infancy and an emerging avenue for exciting research. The fact that BGs of certain compositions exhibit angiogenic potential is an essential finding for further considering BGs in soft tissue repair strategies.

3. Cardiac tissue regeneration

Blockage of the coronary arteries that irrigate the heart results in cardiac ischemia and subsequent tissue death and scarring. This phenomenon is known as myocardial infarction (MI) and is one of the leading causes of death worldwide. Depending on the effectiveness and immediacy of treatment following an episode of MI, the severity of the ischemic injury can be alleviated and swift medical intervention can considerably reduce cardiac tissue damage and its associated pathophysiology [93]. However, in most cases (around 90% of patients) MI causes cardiac arrhythmia, leading to a decreased pumping efficiency that may be life threatening, depending on the extent of the injury. Cardiac tissue lacks the ability to regenerate after definitive damage (around 6 h after MI), thus tissue engineering using injectable scaffolds or engineered cardiac patches is being increasingly investigated as a route to regenerate this damaged tissue [94,95].

Polymeric heart patches, for example, have been developed to provide mechanical support to the left ventricle of the heart and to serve as a cell delivery system to repair the damaged tissue [96–98]. With this double purpose in mind, Chen et al. [99] developed an elastomeric composite for heart patches combining poly(glycerol sebacate) (PGS), to provide the necessary mechanical flexible support, and BG nanoparticles, which were incorporated to improve the mechanical properties of the patches and to provide anchorage points for cells to aid their delivery. Nanocomposite samples were prepared at several BG concentrations, ranging from 0 to 10 wt.%. The patches were placed under culture conditions to
evaluate their degradation behavior. PGS undergoes hydrolytic
degradation in culture medium and thus the pH decreases rapidly.
The addition of BG nanoparticles was found to counteract the acid-
ity caused by the degradation of PGS. In terms of mechanical prop-
ties, the Young’s modulus and strain at break of the samples
increased with increasing proportion of incorporated BG particles,
which confirmed the positive effect of using nanoscale BG in com-
bination with biopolymers, as reviewed elsewhere [59]. Mouse
fibroblasts were cultured with the extract media of the materials
that were cross-linked for 2 days, and it was shown that the prolif-
eration of cells was greater on the nanocomposite samples than on
pure PGS. Similarly, human embryonic stem cell-derived cardio-
myocytes (hESC-CM) cultured with extract media demonstrated
decreased cytoxicity of nano-BG-containing samples compared to
pure PGS samples. Similar values of beating rates were mea-
sured compared to hESC-CM cultured in standard culture medium.
These results show that PGS–nano-BG composites possess suitable
mechanical properties and superior biocompatibility, making them
a viable option for the development of palliative treatment of con-
gestive heart failure via the cardiac patch strategy. Clearly, more
research is required to expand the innovative application of BGs
in this highly relevant field. Indeed, the use of mesoporous glass
particles, which can be loaded with bioactive molecules and exhi-
bite a controlled drug delivery capability [45,100–102], could be
considered as a suitable addition to biodegradable polymers to enhance the functionality of the cardiac patches incorporating
local drug and growth factor releasing effect. A scanning electron
micrograph of such a cardiac patch under development is shown
in Fig. 3a, while Fig. 3b shows a schematic diagram of such flexible
polymer matrix composites incorporating mesoporous bioactive
glass particles (with typical pore size in the range 2–50 nm and
surface area of up to 1000 m² g⁻¹) [101] as local drug releasing
vehicles.

4. Wound healing/dressing

Control of severe hemorrhages could decrease the mortality
rate in extreme situations, such as the battlefield, and natural
disaster and accident areas, by increasing the time window for
the injured to be transported to an adequate medical facility. Sim-
ples palliative wound dressings are often inadequate for the treat-
ment of patients in such cases, and novel biomaterial solutions
have been devised to improve treatment [103]. Thus the search
for biomaterial systems for wound healing involving internal and
external wounds is a continuous task. Bioactive glasses are being
considered for this important application. For example, Ostomel
et al. [104] produced mesoporous BG microspheres (MBGMs) by
the sol–gel method with diameters ranging from 100 nm to 1 μm
for application as hemostatic agents. The increased surface area
of these particles resulted in faster deposition of hydroxyapatite
(HA) on the surface of the MBGMs when immersed in SBF com-
pared to irregularly shaped melt-derived BG particles, and this
effect has been confirmed to positively influence bioactivity
[105,106]. By adding different amounts of MBGMs to sheep blood,
the hemostatic effect of the microspheres was examined, and it
was found that an increasing amount of the spheres decreased
the clotting time, i.e. the rate of coagulation of blood increased in
contact with MBGMs [104]. Although it is unclear whether the
accelerated rate of blood coagulation is due to the increased avail-
ability of Ca²⁺ ions or due to the increased surface area of the cal-
cium-containing BG, the results indicated that MGBMs are
promising hemostatic agents and this field is attracting increasing
research interest. For example, mesoporous silver-exchanged silica
spheres enriched with calcium (AgCaMSS) with diameters in the
range of 600 μm to 1.2 mm were produced by Dai et al. [107]
to investigate them as hemostatic agents with antibacterial proper-
ties. In vitro experiments showed that the addition of Ca and Ag

Fig. 3. (a) Scanning electron microscopy image of a PGS-BG composite developed for cardiac tissue engineering (courtesy of Dr. R. Rai, Erlangen); (b) schematic diagram showing biopolymer matrix incorporating drug-loaded mesoporous bioactive glass particles with (c) dual therapeutic ion and drug delivery ability.
ions into the silica network increased the dissolution rate and weight loss in Tris–HCl buffer solution, and that the optimal formulation of AgCaMSS increased blood clotting rates. Also, incubation of the spheres in a bacterial broth containing *Escherichia coli* and *Staphylococcus aureus* demonstrated the antibacterial effect of AgCaMSS. In vivo studies [107] involved application of the silica spheres to open wounds in the femoral arteries and livers of rabbits, and showed that animals treated with the AgCaMSS agents had lower mortality than controls, which were treated with just conventional gauze and pressure. The hemostatic agents also exhibited low exothermic effects, thus highlighting them as promising materials for hemorrhage control.

In diseases such as diabetes, the wound healing ability of an individual is highly compromised, resulting in longer healing times, increased discomfort and greater risk of infection [108]. The reduction of scarification and faster healing times are also desirable features for a wound healing accelerating device, and certainly there are studies where inorganic materials have been investigated. For example, Majeed and Al Naimi [109] applied HA in a rabbit wound model and found that it promoted faster proliferation of connective tissue and thus enhanced wound healing; in another study, Kawai et al. [110] administered calcium-based nanoparticles to wounded mice and found that the open wound size decreased dramatically in treated animals. The authors speculate that the nanoparticles increased calcium uptake in fibroblasts and caused contraction of collagenous tissues populated with these cells. However, the application of BGs remains an attractive option to be explored in the field of wound healing, given the huge versatility BGs present in the range of ions that can be incorporated in the glass matrix to elicit specific cellular responses [18].

To examine the effects of particulate (<20 μm) BG in full thickness skin wounds, Gillette et al. [111] applied BG into open wounds surgically made in nine dogs. Bilateral wounds were made so that one side could be treated with BG and the other would serve as a control. Before suturing, particulate BG was mixed with a small amount of blood to produce a slurry, which was then applied to the wound. Most of this mixture stayed in the subcutaneous area of the wound, with a smaller amount resting between the wound edges. Laser Doppler perfusion imaging was used to examine changes in blood perfusion around the wound areas, and it was shown that there was no significant difference between treated and control wounds at days 3 and 5. At day 5 post-operatively, the skin and subcutaneous tissue around the wounded areas were excised and placed in a tensiometer to determine the breaking strength of the healed tissue. Whilst no significant difference was found in the breaking strength of the healed skin in all samples, there was an increase in the breaking strength of the healed subcutaneous and cutaneous trunci in the bioactive glass treated wounds compared to the control wounds. It was also found that BG does not cause an increased inflammatory reaction in wounds and could potentially be used to increase the strength of the damaged soft tissue.

The application of nanoscale bioactive glasses in wound healing approaches has also been explored. For example, nanoporous bioactive glass containing silver (n-BGS) was fabricated by Hu et al. [112] to evaluate its performance as hemostatic and antibacterial dressings compared to bioactive glass without nanopores (BGS). Both the n-BGS and BGS samples were prepared by the sol–gel method, with silver contents ranging from 0.01 to 0.04 wt.%. These particles were shown to have an average surface area of about 450 and 91 m² g⁻¹, respectively, with the nanopore samples having a mean pore diameter of 6 nm. As a result of the increased surface area, the water absorption efficacy of n-BGS was much higher than that of the normal BGS. In terms of silver release, the nanopore samples were found to release Ag ions faster, although the concentration of silver in solution was the same after 24 h of incubation in phosphate-buffered saline. *Escherichia coli* bacteria were cultured with n-BGS to test the material antibacterial properties. While samples at all concentrations exhibited antibacterial activity, n-BGS at 0.02 wt.% silver concentration was found to have an antibacterial rate of 99% after an incubation time of 12 h. This glass was chosen for further in vivo experiments since higher Ag concentrations were not considered relevant as they would cause cytotoxicity. The hemostatic performance of the n-BGS samples was measured by both prothrombin time (PT) and activated partial thromboplastin time (APTT) in vitro, and also by applying them to fatal injuries damaging femoral arteries and veins of male New Zealand white rabbits. As an example of the results obtained, Fig. 4 shows hemorrhage control in a rabbit skin injury model treated with n-BGS and BGS particles [112]. In all experiments, the n-BGS outperformed the BGS samples, with significantly lower clotting times in the PT and APTT assays and bleeding times of the rabbit injuries. The findings also demonstrate that nanoporous silver-doped BGS treatments accelerate clotting, facilitate hemorrhage control and exhibit a satisfactory bactericidal effect. In a study by Jebahi et al. [113], ovariectomized rats had their dermal and muscular wounds treated with both plain 46S6 (BG) (see Table 1 for composition [72]) and strontium-containing 46S6 (BG-Sr), and it was found that, compared to untreated control groups, the treated animals exhibited regenerated and well vascularized muscle tissue and fully epithelialized skin. The extent of the healed wounds was more evident in the rats treated with the BG-Sr due to a protective effect against reactive oxygen species from the implants, which the authors speculated was due to ion release from the BG in their surroundings.

With the aim of accelerating the healing of full-thickness skin wounds, Lin et al. [114] produced Vaseline-based ointments with...
18 wt.% of sol–gel bioactive glass 58S (SGBG-58S), nanoscale bioactive glass (NBG-58S) and melt-derived 45S5 bioactive glass powders, which were applied to superficial injuries in healthy and diabetic rats. The healing rates of the wounds treated with the different ointments were compared. After creating full-thickness skin wounds in the diabetic and healthy rat groups, the bioactive glass-containing ointments and Vaseline for the control group were applied directly on the wound site, covered with sterilized elastic bands and reapplied every other day. Wound healing was studied by calculating the healed area as a percentage of the total wound every 2 days up to 16 days and it was found that, for both the diabetic and the healthy rats, healing was accelerated in the presence of the bioactive glasses, most notably with the SGBG-58S, with almost complete wound healing at day 16. Fig. 5 shows the evolution of the treated and untreated full-thickness skin wounds in rats from day 0 to day 16 [114]. The control groups had significantly longer healing times and the wounds were still open by day 16, and, as expected, the diabetic rats had longer healing times than the healthy rats. Histological examination of wound samples demonstrated an increased proliferation of fibroblasts, increased formation of granulation tissue and development of new capillaries in animals treated with the BG-containing ointments, whilst immune-histochemical assays showed that VEGF was present in all tissue samples at day 7. No inflammatory reaction was observed in the animals treated with the bioactive glass ointments. These results demonstrate that bioactive glasses can accelerate wound healing in both normal and diabetic rats. The sol–gel-derived glasses led to quicker and more effective wound healing than the melt-derived 45S5 Bioglass®, which was ascribed to the larger surface area and surface nanoscale topography of the sol–gel glasses.

Similarly, Mao et al. [115] produced Vaseline ointments containing 16 wt.% 45S5 Bioglass® (particle size <53 μm) and different amounts of Yunnan Baiyao, a traditional Chinese medicine of herbal origin, and applied them to full-thickness skin wounds on diabetic rats. It was found that the combination of 45S5 Bioglass® and 5 wt.% Yunnan Baiyao powder significantly accelerated the wound healing rates, and the authors suggested that this phenomenon was due to the combined anti-inflammatory effect of the Yunnan Baiyao and the increased release of relevant growth factors, namely bFGF and VEGF, due to the presence of the 45S5 Bioglass®. It is speculated that these actions resulted in increased fibroblast proliferation and activity, vascularization and granulation tissue growth, thus supporting enhanced healing rates in diabetic rats.

The process of wound healing is complex and involves several stages, starting with an inflammatory reaction that is mediated by several chemical cues, including growth factors and cytokines [116]. It is thought that the ionic products of the reaction of biodegradable BG particles with their surrounding medium induces a reduction of this initial inflammatory reaction, which can increase wound healing rates and thus results in more satisfactory healing [17]. This effect was evidenced in a study by Grotheer et al. [117], in which ortho-silicic acid-releasing silica gel fiber fleeces were implanted in full-thickness skin wounds in pigs. Wounds treated with the fleeces exhibited significantly faster healing rates compared to the controls (in which the wound was simply covered with gauze and plasters).

Dramatic results have been obtained for the repair of chronic wounds in a small-scale human study of “worst case patients” reported recently [118]. Bioactive 13-93B3 (borate) glass nanofibers of composition (wt.%) 5.5% Na₂O, 11.1% K₂O, 4.6% MgO, 18.5% CaO, 3.7% P₂O₅ and 56.6% B₂O₃, with diameters ranging from 300 nm to 5 μm, were used to treat chronic wounds in diabetic patients [118,119]. It was found that wounds healed at an

![Fig. 5. BG treated and untreated wounds from days 0–16 according to Lin et al. [114]. Wounds were treated with different types of BGs, as indicated. It can be seen that SGBG-58S led to the fastest healing rates [114]. Reproduced from ref. [114] by permission of IOP Publishing. All rights reserved.](image-url)
accelerated rate, such as could otherwise be achieved, for example, by applying a vacuum-assisted closure system, which is a relatively expensive and cumbersome process. In addition, there was a marked decrease in scar tissue formation compared to conventionally treated wounds, thus offering a possibility for the treatment of full-thickness skin wounds without lengthy hospital stays or expensive equipment. The success of the limited trials has led to expanded use of the bioactive borate glass fiber wools in patients that otherwise would be candidates for amputation [119].

Given the hemostatic properties of BGs, discussed above, Rai et al. [120] produced poly(3-hydroxyoctanoate) composite films containing nanosized bioactive glass (nBG) to be used as multifunctional wound dressings. Composite films were prepared from 5 and 10 wt.% polymer solutions containing different nBGs concentrations. An increasing proportion of glass nanoparticles imparted the films with increased surface roughness, enhanced the wettability of the polymer and decreased the clotting time of citrated whole blood. In vitro cultures of human keratinocytes on the nanocomposite films showed increased cell proliferation compared to plain polymer films, which the authors attributed to the increase in the number of attachment sites for cells provided by the nBG particles.

When there is extensive tissue loss due to injury, the healing process is delayed, given that tissue replacement takes place simultaneously to repair mechanisms. To aid the progress of these parallel processes, Keshaw et al. [121] produced polyglycolic acid (PLGA) and BG composite microporous spheres with an average diameter of 1.82 ± 0.01 mm by a thermally induced phase separation technique. This approach exploited the bioactivity and angiogenic potential of BGs (discussed above) [21,79,122] and the suitable mechanical properties of PLGA combined with the advantage of the geometry of microspheres. The dimensions and shape of these composite scaffolds make them particularly attractive as fillers for treatment of extensive wounds, as irregular volumes can be packed with these composite microspheres, aiding new tissue growth. In vitro testing showed that composite spheres containing 10 wt.% BG decreased in size by 20% in 16 weeks and induced increased VEGF secretion by human fibroblasts, attributed to the presence of BG (as discussed above). In vivo implantation in rats showed good tissue growth and vascularization around and into the scaffolds. In a similar setting, Day et al. [79] produced meshes from polymer fibers coated with Bioglass® particles that could be applied in a wide variety of soft tissues to aid repair and regeneration (see Fig. 2). Meshes implanted subcutaneously into rats and left for up to 42 days were removed and examined to show complete tissue infiltration and neovascularization, demonstrating the potential of these scaffolds in wound healing applications. Chen et al. [123] produced nanofibrous gelatin/bioactive glass sponges coated with hyaluronic acid and chitosan (NF-GEL/BHA-CS) to treat chronic non-healing wounds, and found that they possessed superior thermal and structural stability compared to plain NF-GEL/BG hybrid sponges whilst maintaining bioactivity, as evidenced by the inorganic ion release when immersed in buffer medium. Thus BG-containing hybrid sponges are an attractive option to accelerate wound healing.

Clearly, with the increasing need for advanced material systems for effective and rapid wound healing, further investigations involving novel chemical compositions and morphologies of BGs, including mesoporous BGs [37,38,46], are required to achieve optimal rates of soft tissue regeneration. Research in this field is likely to increase in the near future, with BGs representing a valid alternative to conventional approaches as a consequence of stimulation of multiple routes of tissue repair while providing a biomechanically attractive delivery system.

5. Nerve regeneration

Peripheral neuropathies resulting from severe injuries cannot always be treated by suturing, and, even in cases where suturing is appropriate, they may result in limited nerve function and sensory deprivation of the affected areas. The gold standard for the treatment of damaged nerves that present a long gap (>4 mm) [124] in these conditions is the use of autografts. However, due to limited availability and possible secondary effects, including donor site morbidity, the need for alternative routes for nerve repair is evident.

Alternative routes for bridging nerve gaps include the use of tubular conduits made from natural or synthetic materials. These biomaterial-based approaches have so far yielded less satisfactory responses than autografts and more research is required. The application of bioactive glasses in nerve regeneration approaches requires in principle the availability of flexible bioactive glass fibers. In view of this, Bunting et al. [125] produced a nerve regeneration scaffold by entubulating 4555 Bioglass® fibers within silastic conduits and tested them by bridging 0.5 cm sciatic nerve gaps surgically created in adult rats. Male Wistar rats were divided into four groups: group A rats had conduits filled with Bioglass® fibers implanted between the nerve stumps; group B had empty conduits; group C had a nerve autograft; and in group D the nerve was severed but left untreated. Groups B–D were used as controls. In groups A, B and D there was a tissue bridge between the nerve stumps, and regenerating axons grew in mini-fascicles. Transmission electron micrographs of 10 µm tissue sections showed that in group A the mini-fascicles were surrounded by connective tissue and were well irrigated by blood vessels. Immune labeling of S-100-positive Schwann cells and neurofilament-positive axons was used to quantify the axonal regrowth between the nerve stumps, which was found to be greater in groups A and C, with comparable results between these two groups. Reinervation was found to be significantly higher in the conduits containing BG (group A) compared to groups B and D [125].

Another approach to produce neuronal guidance constructs was explored by Kim et al. [126] by rolling phosphate glass microfibers (PGFs) into compressed collagen. The efficacy of these conduits was tested in vitro by seeding dorsal root ganglion (DRG) cells harvested from adult Sprague-Dawley rats on the conduits and in vivo by bridging the surgically severed sciatic nerve in these animals. Compared to the controls, the nerve conduits containing PGFs showed increased neurite growth, both in length and number, and faster recovery of motor control in vivo. Histological examination of the composite constructs implanted into the severed sciatic nerve showed axonal extension along the scaffold, whereas the control group did not. Another relevant study, by Novajra et al. [127], involved phosphate glass hollow fibers filled with a genipin-crosslinked agar/gelatin hydrogel and a neurotrophic factor solution. The release profile under culture conditions and the effects that dissolution products of the release system had on the neonatal olfactory bulb ensheathing cell line were investigated. The results showed that there were no deleterious effects on cell growth or expression of pro- and anti-apoptotic proteins in vitro tests, and the use of the hydrogel significantly reduced the release rate of the neurotrophic factor from the hollow glass tubes, which translates into an effective and tailorable system for the delivery of growth factors suitable for tissue engineering applications. More recently, chick DRG cells were cultured with 13–93B3 bioactive borate glass (see Table 1 for composition) microfibers embedded in fibrin scaffolds [128]. It was found that the BG microfibers guided axonal growth, as evidenced by neurite extensions along the fiber axis. It was also observed that the
growth rate was comparable to that of pure fibrin scaffolds. Although experiments conducted with glass rods alone showed decreased cell viability due to a possible cytotoxic effect of glass degradation products, possibly related to the increase in pH of in vitro cultures with a mix of cells (neurons, glia and fibroblasts), it was found that the proportion of live neurons was significantly higher at the end of the experiment in comparison to control dishes.

In a study by Koudoh et al. [129], composite nanoactive glass/gelatin (BGGG) conduits for peripheral nerve regeneration were developed and implanted in rats with severed sciatic nerves. The contractility of the gastrocnemius muscle was examined at 1, 2 and 3 months post-operatively and compared to normal rats. The BGGG group exhibited statistically equivalent nerve regeneration rates. To compare the adhesion, proliferation and differentiation of SKNBE neuronal cells on BGs, Sabbatini et al. [130] cultured these cells on Zn-doped BGs at concentrations of 5, 10 and 20% and compared them to plain 45S5 Bioglass®. The results showed that there was an increase in adhesion and proliferation of undifferentiated SKNBE neuronal cells on the low concentration Zn-doped BGs, whilst proliferation was inhibited at the highest concentration but the genes responsible for the phenotype of differentiated SKNBE cells were up-regulated. The importance of the chemical composition of the BG used was highlighted, which indicates that different bioactive glass compositions may be required, depending on whether decellularized or seeded scaffolds are used. In this context, the evaluation of Zn²⁺ and Ca²⁺ ion release of Si–Na–Ca–Zn–Ce glasses for potential application in nerve guidance conduits has also been carried out [131].

The emerging results discussed here suggest that there is potential for the use of BG fibers in nerve regeneration and that guided axonal growth can be achieved by employing these fibers in nerve conduits. However, improvements must be made with regard to the material choice for the tubular conduit that houses these fibers, and the amount and chemical composition of the BG employed. Another important goal is to compare the performance and cellular mechanisms of nerve response to silicate, phosphate and borate glasses in different constructs, e.g. combined or not with a biopolymer, in their ability to regenerate nerves in controlled in vivo conditions.

6. Gastrointestinal regeneration

Gastric ulcers are caused by a range of factors, including bacterial infection and excessive stomach acid production, and they can be treated with drugs such as omeprazole and hydrotalcite [132,133]. However, given the long recovery time necessary for healing gastric ulcers, there is an increased risk of side effects and toxicity arising from prolonged drug exposure. Very recently, 45S5 Bioglass® has been considered in the context of gastric ulcer healing. To investigate the protective effects of 45S5 Bioglass®, Ma et al. [134] carried out a series of experiments in rats. In the first study, a single oral dose of 45S5 Bioglass® powder (mean particle size 20 μm), omeprazole and hydrotalcite were administered to the animals prior to subjecting them to water immersion stress for 15 h, which produces gastric erosions. These single dosages proved to have a protective effect against gastric ulcers since all treated groups showed that the appearance of lesions was inhibited in a dose-dependent manner. Chronic ulcers induced in another group were treated with 45S5 Bioglass®, omeprazole and hydrotalcite, and it was found that single daily and multiple daily doses increased gastric ulcer healing and protected rats from recurring ulcer flare-ups, also in a dose-dependent manner. To test whether the Bioglass® was orally absorbed, rats were fed 3 mg kg⁻¹ day⁻¹ for 7 days and plasma from blood samples from days 1 and 7 was measured for Si content. There were no significant changes to the amount of Si present in circulation after ingestion for 7 days. This result indicated that Bioglass® particles are not orally absorbable and provided similar ulcer healing rates to two commonly used drugs, with the important advantage of providing a protective effect even after a single dose. Clearly substantial more research in relevant in vivo models is required to confirm the effect of BG in this field.

The application of bioactive glass in intestinal tissue regeneration has been also considered [135]. The intestinal lumen is a highly dynamic environment, where, due to the transit of food and chemical erosion from substances involved in digestion, there is a rapid turnover of epithelial cells [136]. When the superficial layer of the intestine is damaged, leading to superficial wounds, the recovery rate depends on the migration of intestinal epithelial cells to cover the wounded area and a fast cell migration rate reduces the chances of further damage to the tissue. Moosvi and Day [137] tested the effect of 45S5 Bioglass® (mean particle size 2 μm) in epithelial cell restitution by carrying out a series of intestinal epithelial cell and myofibroblast cultures and co-cultures in the presence of Bioglass® particles or conditioned media. A wound model was investigated where a monolayer of intestinal epithelial cells was damaged using a razor blade and the cell migration across the wound bed was observed by measuring the area re-covered by cells after 24 h. It was found that, whilst culture of wounded epithelial cells in medium conditioned with 0.01 and 0.1 wt./vol.% Bioglass® showed no significant increase in cell migration, 1 wt./vol.% conditioned medium increased cell coverage across the wound area by around 50%. This effect was potentiated when the medium was conditioned by Bioglass® and myofibroblasts, and it was most evident with a glass concentration of 0.1 wt./vol.%. In addition, the direct co-culture of myofibroblasts and intestinal epithelial cells in the presence of Bioglass® particles at this concentration yielded the highest wound area recovery rate. Total cell number assays demonstrated that there were no significant changes in the number of viable epithelial cells cultured with medium conditioned by myofibroblast and Bioglass® particles, thus the increased wound cover was attributed to the increased cell migration rate and not to proliferation. The study provided evidence that 45S5 Bioglass® can increase epithelial cell migration in vitro, and thus could potentially help accelerate intestinal epithelium restitution. The application of 45S5 Bioglass® particles added to PLGA matrices in the construction of tubular scaffolds for intestinal regeneration has also been investigated [135]. It was found that the scaffolds possessed satisfactory mechanical properties for this application. Pre-conditioned medium obtained from incubation of tubes with 1 wt.% Bioglass® particles in cell culture medium (Dulbecco’s modified Eagle’s medium supplemented with 2 mM glutamine and 1% penicillin & streptomycin) was used to culture mouse fibroblast cells. There was a considerable decrease in cell population compared to controls, on average 32%. However, the morphology of the tubes, with diameters in the range from 1.5 to 3 mm and interconnected porosity, was reported to make them interesting options not only for gastrointestinal applications, but also other applications where tissue-like tubular structures are required. The experimental results indicated that the critical combination of bioactive glass particles and biopolymer matrices with a tubular shape could be used for the regeneration of tube-shaped organs. A biodegradable mesoporous structure of BG particles to deliver biomolecules [101,138] would be an ideal medium for incorporating a local drug delivery function to the scaffold (Fig. 3). Such highly biodegradable, multi-functional 3-D mesoporous BG architectures could be used to “tune” the composite scaffold degradation behavior to synchronize it with the specific cellular changes taking place in soft tissue regeneration.
7. Urinary tract

Urinary incontinence and vesico-urethral reflux result from insufficient urethral resistance and are major health complications in aged patients, leading to a very poor quality of life and great expense in later years. A number of approaches to tackling these conditions have used injection of diverse materials into the bladder neck to increase continence and to avoid the risks associated with open surgery [139]. Polytetrafluoroethylene (Teflon) and collagen are commonly used materials in these cases, but there are potential risks associated with particle migration and loss of volume with time which could compromise safety and effectiveness. Stimulated by the seminal discovery of Wilson et al. [65] of strong, stable bonding of soft connective tissues to bulk and powder 45S5 BG samples, Walker et al. [140] began investigating BGs as alternatives to the non-adherent bioinert particle suspensions for treatment of incontinence. Suspensions of 45S5 and 45S5F (composition (wt.%) : 45% SiO2, 5.5% Na2O, 12.25% CaO, 6% P2O5, and 12.25% CaF2) bioactive glasses in sodium hyaluronate were tested as urethral bulking agents in two experiments. The first experiment involved injecting 0.1 ml of the BG suspensions in the bladder dome of 12 rabbits and analyzing tissue samples from 2 to 12 weeks for inflammatory reaction or particle migration. Histological examination of bladder samples at the injection site showed that the particulates were surrounded by collagen fibers and connective tissue. No inflammatory response was detected for any of the BGs used. Importantly, chemical analysis did not detect any increase in calcium or silicon content in lung, liver, kidney and lymph nodes. The second Walker and Wilson experiment involved injecting 1 ml of 45S5 Bioglass® suspension into each of the four quadrants of the bladder neck of four pigs and measuring the urethral resistance pre- and post-operatively with urethral pressure profiles. Three months after the injection, there was an increased maximal urethral pressure, indicating that there was an increase in urethral resistance in all animals; however, the significance of the experiments could not be tested because of the small size of the test group. Histological examination of injection site samples indicated that all BG particles were well integrated into the surrounding connective tissue and there was no evidence of an inflammatory reaction. The need for a safe and injectable material that is bioactive and forms strong, stable bonds with the surrounding tissues is evident, and the study [140] showed that BGs can fulfill both of these criteria. Further research in this area is required, which should involve in vivo tests in a functional animal model with a sizeable group for statistically significant assessment of the efficacy of this mode of treatment.

8. Lung tissue engineering

Adult lung tissue exhibits poor regenerative capabilities and in cases where there is extensive damage, e.g. with diseases like cystic fibrosis or COPD, the only option for patients is a full heart and lung transplant [141]. Tissue engineering of the lung is being explored using various biomaterials [142–144]. BGs have been also considered in this application. Verrier et al. [145] produced composite foams based on PDLLA and 45S5 Bioglass® (with a mean particle size <5 μm) exhibiting interconnected pores. The foams were seeded with human lung-derived immortal cells to assess their suitability as tissue engineering matrices. In vitro tests indicated that cell adhesion and proliferation were strongly dependent on the Bioglass® content of the foams. The highest cell proliferation occurred in scaffolds containing 5 wt.% Bioglass®, and cell adhesion increased with increasing Bioglass® particle content. All scaffolds studied showed full cell penetration, even in the absence of Bioglass® particles.

In a study by Tan et al. [146], sol–gel-derived BG foams with surface modifications to include amine or mercaptan groups and/or coated with laminin were manufactured and placed in culture with murine lung epithelial cells (MLE-12) to determine the best conditions to promote growth and proliferation. Based on histological examination of the cell cultures, there was full penetration of the foams by the lung cells and it was shown that the laminin-coated, amine-modified foams were most effective in promoting cell growth and attachment. These results seem to indicate the possibility of using BGs in lung tissue engineering approaches, although extensive work, including testing with the different cell types found in this complex tissue, is necessary for further advancements. At present, the role of the bioactive phase in promoting lung cell proliferation and/or differentiation is as yet unknown. Basic cell and molecular biology studies of potential for bioactive stimulation of type 2 pneumocytes for lung tissue regeneration are still to be done, and much research selecting appropriate glass compositions and morphologies, e.g. combined with suitable soft and porous polymer matrices, is needed in order to realistically consider bioactive glasses in this particular field.

9. Laryngeal repair

Patients with problems secondary to paralysis or surgery of a cancerous vocal cord have a weak, breathy voice. More importantly, such patients may also have problems due to inefficient air use and potential for liquid aspiration. Effective treatment is movement of the paralyzed cord so that it approaches the normal cord sufficiently to improve speech and prevent aspiration down the airwaves. Previous clinical solutions have used an injectable form of PTFE or the surgical insertion of silicone rubber. Serious concerns with these treatments include deterioration of the voice and potential release of dangerous emboli. The complication of extrusion into the airway is a particular concern. A material which does not extrude into the airway and which permits some degree of customization of the repair and long-term stability is needed.

Particulate bioactive glass (45S5 composition) was investigated for vocal cord medialization because of its proven ability to bond to soft tissues and the absence of migration or adverse effects due to strong stable bonding to collagen, as reviewed recently by Hench and Greenspan [17]. Their review presented an investigation of 45S5 Bioglass® particulates in a canine model conducted by the team of Wilson, Slattery and Crary (University of Florida). The implantation of bioactive glass particulates improved the bark characteristics over those of the paralyzed vocal cord. The characteristics of the dog bark post-implantation and repair were similar to those of normal larynx, suggesting increased laryngeal valving during phonation. The histological analysis showed that the particulate was in place between the thyroid cartilage and the vocal cord. Most of the particulate was contained in adherent soft tissue. In several areas, bony metaplasia, which is characteristic of the normal repair of this type of cartilage, had enclosed bioactive glass in bone and cartilage as well. The conclusion from this study is that implanted bioactive glass powder can be used to produce sufficient medialization of a paralyzed vocal cord to produce improvement in phonation. The tissue augmentation which produces this effect is the combined effect of the immobilization of bioactive glass by the combination of hard and soft tissue in the bony metaplasia, which is part of the normal cartilaginous repair process. The study provided an example of the importance of collagen–bioactive glass adherence and the long-term stability of such a bonded interface in a functioning animal model [17]. Optimization of the bioactive glass particulate delivery system to ensure accurate placement of the ideal quantity and size of particulate is still to be studied.
10. Stabilization of percutaneous devices

Catheters are widely used in healthcare, especially for aged patients, such as peritoneal dialysis catheters and indwelling catheters for treatment of urinary incontinence. Catheters typically develop a thin, non-adherent fibrous capsule around the device. They thus lack a mechanical barrier to periluminal bacterial migration and need cuffs for anchorage. The space between the device and the interposed fibrous layer can provide a pathway for infections to spread from the percutaneous site, with serious consequences to the patient. Continual monitoring and maintenance of catheters is a growing burden to the healthcare community and innovative approaches to solving this problem are greatly needed. Technology developed by Marotta et al. [147] showed that it is possible to produce stable, adherent bioactive glass coatings on silicone catheters and other silicone medical devices and to achieve collagen bonding to the bioactive surface, which can stabilize the devices in soft tissues. The feasibility of using bioactive coatings on silicone for tissue adhesion of peritoneal dialysis catheters was reported by Ross et al. in 2003 [148]. Segments of 45S5 bioactive glass coated silicone tubes, 2.5 cm long, were implanted in rats, with uncoated tubes as controls. Histological analysis of the tissue–implant interfaces was conducted at 2, 4 and 6 weeks using special stains and electron microscopy. Results showed that "uncoated tubes had no adherence to the surrounding tissues and had a physical separation of approximately 50 micrometers width comprised primarily of acellular collagen. In contrast, the bioactive glass coated tubes were palpably fixed to the soft tissues" [148]. The authors summarized that the BG coatings improved tissue retention of the silicone tubing as they promoted adhesion by collagen bonding to the bioactive surface, which can stabilize the catheters and other percutaneous devices due to the increasing clinical need.

11. Discussion

11.1. Bonding mechanism between BGs and soft tissues

The mechanism of bonding between bioactive glasses and tissues is thought to be governed by the time-dependent dissolution and precipitation reactions that occur at the implant interface upon contact with physiological fluids. These reactions cause a local increase in the concentration of various ions at the BG surface, resulting in an overall increase in pH and the formation of a carbonated HA (HCA) layer. This crystalline layer provides the bonding interface between the implant and the aforementioned tissues, and, as mentioned in the Introduction, has been the key aspect that has been investigated over the years to rationalize the bone bonding ability of bioactive glasses [4,8,12].

It is now well accepted that there are two kinds of interactions that occur in the body upon implantation of BGs [19]:

1. Extracellular interactions that are determined by the specific surface characteristics of the material, which in turn govern protein adsorption and subsequent cell attachment.
2. Intracellular interactions between the protein ligands formed on the implant surface and cell receptors that determine the degree of cellular adhesion, differentiation and proliferation.

These two types of interaction are affected by the availability of different ions. For example, HA is known to attract macrophages in wounded tissues, evidenced in a study of wound healing in a rabbit model [109]; local delivery of calcium can enhance tissue regeneration in wounded mice [110] and silica has been shown to promote healing of chronic wounds on mouse ears [149]. Additionally, it has been shown that the collagenous constituents of soft tissues are able to attach to the surface of BGs, although it is worth noting that the degree of bonding and rate of regeneration are dependent on the progenitor cell populations available in the surrounding tissues [150], this being precisely the area requiring further study due to the patchiness of current knowledge. Nevertheless, the available data suggest that BGs enhance tissue regeneration and stimulate tissue attachment to the implant surface, and that this effect is highly dependent on the glass ion releasing functionality and on biomechanical effects.

11.2. Biomechanical factors in soft tissue engineering

Some of the clinical applications of bioactive glasses in contact with soft tissues have two primary requirements for success:

1. Rapid formation of an interfacial bond with collagen.
2. Stable long-term interfacial bonding that prevents micromotion at the interface and the onset of an inflammatory response.

In addition, long-term stability of the implant bonded interface has another requirement, namely:

3. A gradient in stress transfer across the interface that avoids cell signaling to resorb either tissues or the implant.

The excellent long-term clinical success of 45S5 bioactive glass devices for replacement of the bones of the middle ear and implants for maintenance of edentulous jaws (ERMI), reviewed by Hench and Greenspan [17], are attributed to satisfying all three of the above requirements for both bone and soft connective tissues. Rapid formation (within weeks) of the interfacial bonding layers and fast regeneration of new bone at the implant–bone interface make BG implants stable and ensure proper stress transfer to the stapes footplate or alveolar bone to prevent the onset of bone resorption. Stable bonding of the implant to soft tissues prevents extrusion of the ossicular replacements through the tympanic membrane or exfoliation through gingival tissues. A study in dogs conducted by Wilson et al. [151], using the same ERMI implants, burs and protocol as for humans, made it possible to achieve a quantitative histo-morphometric analysis of the hard and soft tissue bonding interfaces of the ERMIs. Within 3 months, the bonding had stabilized for both hard and soft tissues. The soft tissue was bonded by collagen fibers interdigitated within a 150–400 µm thick bonding gel layer composed of biological HCA and an underlying silica-rich gel layer that began to form on the implants within minutes of implantation.

An important finding in the Wilson et al. study was that the thickness of the soft tissue bonding layer (Fig. 6A) was nearly 50% thicker than the bonding zone between the bone and the implant (Fig. 6B). The thickness of both bone and soft tissue interfaces was stable after about 7 months, as shown in Fig. 6 [151]. Finite element modelling of the bioactive glass tissue interfaces by Weinstein et al. [152] provided an important insight into the effect of elastic modulus mismatch on the success of implants bonded to either hard or soft tissues. Fig. 6A (top right) plots the gradient of stiffness (Young's modulus) between a soft tissue interface and a bulk bioactive glass implant. The difference in modulus is spread over a substantial thickness of interface due to the elastically compliant hydrated silica gel (HCA) layer formed on the bioactive glass, which is several hundreds of micrometers thick. The rapid formation of the bonding layer, as well as the elastic compliance (low stiffness) of this bonding zone and the favorable stress...
transfer resulting from the bonding of collagen fibrils, is concluded to be responsible for both the short- and long-term success of 45S5 Bioglass ERMIs [151,152]. In contrast, synthetic bioceramic HA implants bond much more slowly and form a bond only to bone, with a bone–implant bond thickness of <1 μm. This thin bonding interface of HA implants results in a much greater (by a factor of >400) gradient of elastic modulus between the living and non-living materials. The unnatural gradient in the elastic modulus of HA implants results in large stress gradients to the bone and stimulates resorption and failures of the alveolar ridge or maxillofacial and cranial bones, as described in the literature [17].

The biomechanical behavior of clinical applications that involve interfacial bonding of bioactive glass particulates or fibers to soft tissues or in situ regeneration of soft tissues, as reviewed above, is unlikely to be as important as for bulk bioactive implants. However, it is important that a systematic study be conducted to investigate the effects of various volume fractions, sizes and shapes of bioactive glass particles and fibers on the elastic properties of augmented and regenerated soft tissues that contain bioactive phases. Long-term cyclic fatigue effects that can lead to the breakdown of the particle–tissue interface must be avoided for the long-term survivability of such clinical applications.

12. Conclusions

The range of applications for BGs in contact with soft tissues is vast, and the specific biochemistry of BGs in relevant environments both in vitro and in vivo has been shown to stimulate regenerative processes such as wound healing and angiogenesis. Further investigations of the specific molecular biological mechanisms involved in the regulation of these processes and how they are affected by the ionic products that result from the dissolution of BGs when implanted into soft tissue are necessary to fully unlock the potential that these inorganic materials offer to regenerative approaches in tissues other than hard (bone, teeth) tissues. Fundamental knowledge of the mechanisms involved should also lead to strategies in which the addition of BGs will complement the application of growth factors or other biomolecules, possibly reducing the reliance on such molecules in tissue engineering.

It is clear from the results of the investigations discussed in this review that there is a huge potential for applications of BGs in soft tissue engineering and wound healing. However, there is a need for a significant amount of research to be carried out in order to establish the specific mechanisms involved in the interaction of BGs and the wide range of cells that compose the different soft tissues in the body, just as has been carried out for hard tissue applications. It is crucial to develop constructs for tissue engineering applications that will support cell attachment and proliferation, present suitable mechanical properties and have degradation rates that match the recovery rates of the host tissue, as well as being available in a manner that appeals to surgeons and other clinical staff directly involved in their possible use. Research in this area needs to move beyond current investigations involving co-culture of relevant cells with BG particles and towards incorporation of these materials into 3-D matrices suited to the function and morphology of specific soft tissues, considering the hierarchical structure and complexity of the tissues and their nanotopography and cell cytokine–bioactive material signaling. Biomechanical aspects specifically related to the bonding mechanisms of BGs to soft tissue, which should lead to improved interface behavior (e.g. via collagen-graded structures), must be further quantified by considering additional in vivo tests and quantifying the effect of ion dissolution involving different chemistries (most studies in this area have been conducted using the 45S5 BG composition). The addition of biochemical cues, such as doping ions, growth factors and antibiotics, that may potentiate and support tissue renewal will also be relevant for new regeneration strategies. The addition of BGs in different forms, namely micro- and nanoparticles, fibers, mesoporous structures and hybrid inorganic–organic matrices, is bound to expand in a number of applications, from vascularization to wound healing, and potentially even to organ regeneration and repair.

Fig. 6. Development of reaction layers on Bioglass® ERMi ridge maintenance implants for (A) soft tissue interface and (B) bone interface. The insets are the calculated elastic modulus gradients of the (A) implant–soft tissue interface and (B) bone–implant interface. The figure is based upon results of Wilson et al. [151] and Weinstein et al. [152]. Adapted from ref. [17]. Reproduced with the permission of J. Austr. Ceram. Soc.
These approaches will help us move towards creating a soft tissue engineering construct that takes full advantage of the ideal physiological conditions in the body whilst maximizing the regenerative potential of soft tissues.

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Appendix A. Figures with essential colour discrimination

Certain figures in this article, particularly Figs. 2–5 are difficult to interpret in black and white. The full colour images can be found in the on-line version, at http://dx.doi.org/10.1016/j.actbio.2014.11.004.

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