Bioactive glasses: special applications outside the skeletal system

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Abstract

Bioactive glasses were invented 45 years ago and have been in clinical use since the 1980s in otology, orthopaedics and dentistry. Initially born as bioactive materials to fill bone defects, bioactive glasses expanded their biomedical suitability towards a broad spectrum of tissue engineering and therapeutic applications, and research evolution seems to witness that their potential is far from being fully exploited. Classical applications of bioactive glasses involve bone filling materials and dental implants; however, the fascinating question to be answered in the next few years is: how can bioactive glasses be useful in soft tissue regeneration and to treat diseases, such as tumours, that may affect internal organs? This review paper focuses on research that demonstrates the suitability of bioactive glasses in contact with tissues outside the skeletal system,

including muscle and nerve tissue regeneration, treatment of diseases affecting sense organs (eye and ear), embolization of neoplastic tissues, cancer radiotherapy via injectable microspheres, and wound dressing. A prospect for future research is also provided, highlighting the potential associated to targeted therapy via local ion release, angiogenesis stimulation and *in situ* drug release, as well as the promise of biofabrication for the development of bioactive glass-containing composite constructs for organ regeneration.

Keywords: Bioglass; Composites; Angiogenesis; Antibacterial properties; Cancer treatment.

Highlights

- Bioglasses (BG) can be successfully used in contact with tissues outside the skeleton
- Ion release from BG can be exploited to induce angiogenesis and antibacterial effect
- Biofabrication can allow smart BG/polymer/cells constructs to be tailored

1. Introduction

The idea of replacing damaged body parts in humans by implanting biological or artificial materials dates back to prehistory and there is evidence that, later, ancient Egyptian physicians systematically designed wooden prostheses (aesthetic foot and hand fingers) to help their ailing patients [1]. In the past, the research for implantable biomaterials was primarily addressed to as inert as possible materials that did not interact with biological environment; this concept was revolutionized with the invention of the first bioactive glass, currently marketed under the name of 45S5 Bioglass®, by Hench and co-workers in the late 1960s [2]. Bioactive glasses are, formally, non-crystalline ceramics that are able to bond to living tissues (primarily bone) and to stimulate new tissue growth while dissolving over time: these properties make them valuable candidate materials for tissue engineering applications. Weinstein et al. [3] showed that the strength of the interfacial bond between Bioglass® and patient's bone was equal to or greater than the strength of the host bone. Bioglass® particulate has been in clinical use since 1993 under the commercial name of Perioglas®, used to fill periodontal bone defects, and more recently as NovaBone® (porous granules, injectable pastes, shaped morsels), used for orthopaedic and dental applications. Bioglass® has also been applied clinically in middle ear surgery and in one of the first prototypes of cochlear implants to anchor the device through the patient's temporal bone [4].

Since the initial discovery of 45S5 Bioglass®, many other glass formulations and types have been found suitable for biomedical applications [5-7]. From a compositional viewpoint, bioactive glasses can be basically divided into three groups, depending on the representative former oxide present in the formulation, i.e. SiO_2-based (silicate), B_2O_3-based (borate) and P_2O_5-based (phosphate) systems. The first group comprises a wide range of glass formulations, including 45S5 Bioglass® (46.1SiO_2-24.4Na_2O-26.9CaO-2.6P_2O_5 mol.%); borate glasses are characterized by higher reactivity than silicate materials, which results in faster bioactive kinetics [8]; phosphate glasses are resorbable materials and their dissolution rate can be tuned according to their oxide composition [9].

In addition to SiO_2, B_2O_3 and P_2O_5, various amounts of other oxides may be incorporated in glass composition to impart peculiar properties to the material; for instance, CaO, K_2O, Na_2O and MgO are useful to adjust the surface reactivity in biological environment; ZnO, CuO and Ag_2O allow the release of proper ions with antibacterial properties; Al_2O_3 is helpful to strengthen the mechanical properties [10]. It should be underlined that even a small variation in glass composition can deeply modify the features of the material.

Bioactive glasses are commonly produced by melting-quenching routes or sol-gel technique. Cast glasses can be poured into moulds in order to produce rods, bars or, in general, components of various size and shape (e.g. prosthetic middle ear ossicles) [4]. If produced in form of powder, glass can be used as starting material to fabricate tissue engineering three-dimensional (3-D) porous scaffolds [7]. Glasses with a mesoporous texture can be produced by wet synthesis for possible use as local drug release vehicles [11,12]. Furthermore, these glasses can be drawn or electrospun to obtain micro- or nano-sized fibres [13,14].

Glass conversion into a partially crystalline material can be achieved by applying appropriate heat treatments (e.g. sintering); usually, the resulting glass-ceramics exhibit superior mechanical properties but lower reactivity in biological environment with respect to parent glasses.

Until the early 1980s it was believed that only calcified tissues could form a bond to bioactive materials. Wilson et al. [15] first showed that soft connective tissues could also bond to 45S5 Bioglass® if the interface was immobile. Some glass-ceramics belonging to the family called Ceravital® (SiO_2-P_2O_5-CaO-MgO-Na_2O-K_2O system) could be also incorporated into connective tissues being surrounded by a soft collagenous fibrous membrane with no adverse reaction [16]. Wilson and Noletti [17] assessed the compositional dependence of the bonding of SiO_2-CaO-Na_2O-P_2O_5 bioactive glasses to connective tissues, demonstrating that the material can bond to bone but not to soft tissues when the glass composition exceeds 52 wt.% of SiO_2. This set of data provided the basis for clinical use of Bioglass® in middle ear ossicles replacement, where an implant able to bond both to bone and to tympanic membrane was desirable; since 1985, when this first Bioglass®

device was approved for clinical use, the spectrum of medical applications of bioactive glasses expanded dramatically going well beyond small bone replacement (Fig. 1). In perfect agreement with an impressive sentence by Prof. Hench, who wished that "creative studies of novel glasses and glass-ceramics are needed now more than ever to cope with the problems of a world that has finite resources but infinite desires" [18], today bioactive glasses are also proposed for a wide range of non-osseous tissue engineering applications that seemed impossible when research began. In this regard, emerging fields of research for bioactive glasses include neuromuscular repair (fibrous constructs for muscle and nerve regeneration), artificial cornea, orbital implants, epithelial and cardiac tissue engineering, treatment of gastric ulcers and non-osseous cancer therapy. A recent review paper has covered the applications of bioactive glasses (and their composites with biopolymers) in the field of soft tissue engineering, highlighting their potential in cardiac, lung and gastrointestinal tissue regeneration approaches [19]. In the present work, the clinical and experimental applications of bioactive glasses for the treatment of various diseases outside the skeletal system, excluding those already discussed by Miguez-Pacheco et al. [19], are systematically reviewed (Table 1). Future research directions are also explored and outlined in the light of the recent findings in tissue engineering and biomaterials processing technology.

2. Wound healing: emphasis on the effects of ion dissolution products from bioactive glasses

Wound healing represents a major challenge in medicine and is commonly associated to a number of clinical scenarios including skin regeneration, chronic wounds (e.g. non-healing diabetic ulcers), damage to mucosae and surgical sutures.

The skin plays an important role in the prevention of infections from pathogens and at the same time keeping the homeostasis of the body. Once a trauma is suffered, the damaged skin should be immediately covered with a dressing able to maintain a moderately moist environment for regeneration of the skin, prevent infection, alleviate pain and remove excessive exudates. In an

interesting animal study, Gillette et al. [20] determined the effects of intraincisional bioactive glass particulate on the healing of sutured full-thickness skin wounds in dogs. The presence of glass particulate in soft tissues did not cause a gross inflammatory reaction but induced an increase in histologic signs of inflammation, which decreased with time; furthermore, subcutaneous breaking strength at 5 days was significantly higher in glass-treated wounds than in control wounds, which indicated that bioactive glass could be beneficial in treating wounds in which early healing strength is needed.

The potential of some metallic cations to act as antibacterial agents in the context of wound healing has been widely studied as an alternative to traditional antibiotic treatments which may be followed by bacterial resistance. In this regard, bioactive glasses are a valuable resource as they can be doped with various metal oxides to provide a smart strategy for the controlled delivery of therapeutic ions *in situ* [21]. Several studies investigated the incorporation of gallium (Ga), silver (Ag) and copper (Cu) in silicate and phosphate glasses to prevent infections in wound dressing. It was reported that a concentration as low as 1 mol.% of Ga_2O_3 in a phosphate glass was adequate to impart a potent antibacterial effect due to the sustained release of Ga^{3+} ions [22,23]. Silver has been shown to induce epidermal repair in sterile skin wounds in rats by reducing the inflammatory and granulation tissue phases of healing [24]; released silver ions also exhibited bactericidal action as they damaged bacterial RNA and DNA, hence inhibiting replication [25]. It was reported that incorporation of 3 wt.% of Ag_2O imparted antimicrobial properties to a silicate glass without compromising the material bioactivity [26,27]. Wren et al. [28] described antibacterial (using *Staphylococcus epidermidis* and *Escherichia coli*) and antifungal (using *Candida albicans*) effects *in vitro* for silver-coated glass particles in the $42SiO_2$-15CaO-$23Na_2O$-20ZnO (mol.%) system prepared by dipping the melt-derived particles in $AgNO_3$.

These concepts have been applied to surgical sutures that, incorporating a bioactive and antibacterial phase, can result in multifunctional composite materials with a wide range of applications in wound healing. Blaker et al. [29] developed novel polymer-based sutures coated

with silver-doped bioactive glass powder by slurry dipping method (Fig. 2). Boccaccini et al. [30] noted that Bioglass®-coated Vycril® sutures, produced by this method, exhibited a decrease in tensile strength compared to uncoated ones (385 vs. 467 MPa): the possible explanations were associated to mechanical damage of the suture surfaces by the hard glass particles upon coating preparation [31] and/or the possible infiltration of glass particles into the voids of the braided structure of the suture.

Pratten et al. [32] carried out *in vitro* experiments using *Staphylococcus epidermidis* to investigate the antimicrobial activity of commercial Mersilk® sutures coated with 45S5 Bioglass® powder and a sol-gel Ag-doped glass ($60SiO_2$-$34CaO$-$4P_2O_5$-$2Ag_2O$ mol.%): under batch conditions of up to 180 min, Ag-doped sutures showed a significantly greater effect in limiting bacterial attachment compared to Bioglass®-coated and uncoated sutures. The research group coordinated by Prof. Jonathan Knowles extensively investigated the effect of Ag-doped phosphate glasses on bacterial biofilm formation and growth for *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, as well as how the rate of Ag^+ ion release can affect the bactericidal effect [33-36]. The same research group also studied the antibacterial effect of Cu-doped phosphate glasses against *Streptococcus sanguis* for potential wound healing applications [37] and developed P_2O_5-CaO-Na_2O glass fibres doped with up to 10 mol.% of CuO [38]: it was found an increase of Cu^{2+} ion release from the glasses with higher copper content despite their lower dissolution rate, associated to an increasing reduction of the viable bacteria both adhering to the fibres and present in the culture medium.

Interesting clinical applications of bioactive glasses for the healing of oral mucosa were reported by Stoor et al. [39-41], who investigated the effect of a S53P4 glass ($53SiO_2$-$23Na_2O$-$20CaO$-$4P_2O_5$ wt.%) implants on a wide range of oral pathogens in a series of studies carried out in humans. A S53P4 paste exhibited a potent and relatively fast antimicrobial effect (10-60 min depending on the type of bacteria), inhibiting the viability of microorganisms of both supra- and sub-gingival plaque [39]. S53P4 granules and disks were also used as interpositional implants in 11 patients suffering

from nasal septum perforations [41]; successful closure was obtained in 10 cases and no implant extrusions[1] or infections in the nasal cavity were reported after follow-up periods between 24 and 37 months. The effect of S53P4 on *Klebsiella ozaenae*, a microorganism associated to atrophic rhinitis, was also studied both *in vitro* and in human patients: after follow-up periods between 19 and 74 months, the foul odour disappeared and the mucosal membrane fully normalized [40].

A few commercial products containing Ag-doped phosphate glass with a polymeric adhesive for woundcare film dressing (Antimicrobial Arglaes® film, Antimicrobial Arglaes® Island, Medline) and with alginate for topical powders (Arglaes® powder, Medline) are already on the market and allow a prolonged control of infections.

Angiogenesis is a key phase of wound healing which leads to the invasion of capillaries into the wound clots; acceleration of this process is therefore crucial and can open new perspective for the treatment of such injuries. In this regard, there is convincing evidence that the ion dissolution products released by bioactive glasses, apart from eliciting an antibacterial effect, can promote angiogenesis both *in vitro* and *in vivo* [42]. Day et al. [43] first showed in a series of *in vitro* (with fibroblasts) and *in vivo* (in rats) experiments the ability of 45S5 Bioglass® incorporated into poly(glycolic acid) (PGA) meshes to increase the scaffold neovascularization, which would be highly beneficial for the engineering of large soft tissue constructs. More recently, Lin et al. [44] investigated the effect of bioactive glass ointments, prepared by mixing sol-gel 58S glass ($58SiO_2$-$33CaO$-$9P_2O_5$ wt.%) micro- and nano-powders, melt-derived 45S5 Bioglass® particles and vaseline, on cutaneous wounds in both normal and streptozotocin-induced diabetic rats. In general, the analysis of wound healing rate and wound healing time showed that bioactive glasses promoted wound healing, stimulating the proliferation of fibroblasts and the growth of granulation tissue. The ointments containing glass nanoparticles healed the wounds more quickly and efficiently than those prepared using commercial micro-sized Bioglass® particles only. Immunohistochemical staining showed that the production of the growth factors VEGF and FGF2, which are beneficial to wound

[1] In surgery, the term "extrusion" refers to the expulsion or spontaneous removal of an implant from the host tissue.

healing, was also stimulated during the healing process; fibroblasts in wounds treated with bioactive glasses also contained rougher endoplasmic reticula and formed new capillary microvessels by the seventh day. Nano-sized 45S5 Bioglass® particles were also used by Rai et al. [45] in the fabrication of a novel poly(3-hydroxyoctanoate)-based composite scaffold for wound dressing: the incorporation of bioactive glass nanoparticles accelerated blood clotting time and enhanced the wettability, surface roughness and overall biocompatibility of the scaffold.

Another strategy that has been recently investigated in the literature to promote angiogenesis is the use of cobalt-doped glasses [46], as the release of Co^{2+} ions is known to mimic hypoxia and, accordingly, the creation of hypoxia conditions is suggested to be a strategy for activating pro- and anti-angiogenic genes [47]. This is an interesting finding that can open new frontiers in the use of bioactive glasses for wound healing and skin regeneration; however, the risks associated to cobalt toxicity *in vivo* still deserves careful assessment [48].

In recent years, a few experimental 2-D and 3-D bioactive glass-based constructs have also been developed specifically for skin tissue engineering. Chen et al. [49] reported the successful synthesis of a nanofibrous gelatin/Bioglass® composite hydrogel by phase separation method followed by arming the nanofibres network with counterionic chitosan-hyaluronic acid pairs for improving the microstructural and thermal integrity of the scaffold in wet state. Nano-sized Bioglass®/collagen composite membranes have also been proposed as carriers for the sustained release of tetracycline hydrochloride (a wide-spectrum antibiotic) in the context of skin repair and wound dressing [50]. Hong et al. [51] proposed the use of ultrathin (diameter around 600 nm) mesoporous bioactive glass (MBG) hollow fibres (Fig. 3), fabricated by electrospinning combined with a phase-separation inducing agent (poly(ethylene oxide)), as a multifunctional system for skin tissue engineering (support to the regenerated tissue and release of appropriate drugs) when organized in the form of 3-D macroporous membranes. Drug loading and release experiments using gentamicin sulphate indicated that the drug uptake and release capacity strongly depended on the fibre length; fibres with length above 50 μm were shown to be excellent carriers for drug delivery, whereas the

shortening of the fibre length reduced drug loading amounts and accelerated drug release. MBGs were also mixed with chitosan to produce composite films by freeze-drying for possible use as haemostatic membranes for skin repair [52].

A few experimental studies have demonstrated that bioactive glasses can be exploited to promote the healing of particular cases of internal wounds which affect the gastrointestinal tract. Alkaline ion dissolution products from bioactive glasses can have an antacid effect, thus favouring the healing of gastric ulcers. Interestingly, it was also reported that 45S5 Bioglass® particles can play an active role in the healing of superficial injury of the intestinal mucosa, promoting a process termed epithelial restitution. These emerging applications were reviewed elsewhere in detail [19].

3. Peripheral nerve and spinal cord repair

Peripheral nerve injuries result in the partial or total loss of motor, sensory and autonomic functions in the area of the body concerned. They can be the consequence of a mechanical stress, exposure to heat, cold, irradiation, electrical injuries, burns, tumours and focal inflammation [53]. In the presence of serious tissue damage or a complete nerve transection, the spontaneous regeneration process of the peripheral nerve occurs, mainly involving the migration of Schwann cells along the nerve gap supporting the re-growth of axons from the proximal to the distal stump. However, the regeneration is often not sufficient to provide an adequate target re-innervation and a surgical intervention is then needed. When end-to-end suture of the nerve stumps would generate excessive tension, the use of a nerve autograft or allograft (nerve gaps larger than 5 cm) is the current gold standard, even if these options imply obvious drawbacks and rarely lead to fully satisfactory functional recovery [53,54]. At present, an alternative to the actual grafting techniques is represented by the use of a polymeric nerve guidance channel (NGC) sutured in-between the two nerve stumps, which protects the regenerating nerve from the scar tissue infiltration and allows the soluble factors accumulation to be maximized [54]. The currently commercially available devices

for the treatment of the peripheral nerve injuries are NGC (i.e. tubes) or membranes to wrap around the nerve stumps made of natural (e.g. collagen, porcine small intestinal submucosa) or synthetic polymers (e.g. PGA, poly(DL-lactic acid) (PDLLA)/caprolactone, poly(vinyl alcohol) (PVA) hydrogel) [55].

Glasses have also been studied for nerve regeneration in bulk, powder or fibre form, alone or in combination with a polymeric phase to produce a composite material. The first study that proposed the use of a glass for nerve regeneration was reported by Gilchrist et al. [56], who developed a resorbable phosphate glass tube (length 4 cm, inner diameter 4 mm) for the repair of divided facial nerve of sheep. The two nerve stumps were approximated inside the tube, which was then sutured to the epineurium through the holes at the tube extremities. Ten months after the surgical intervention, the glass tubes fully dissolved and all the nerves were completely regenerated with uniform diameter along the length. The authors concluded that the use of a soluble glass tube could be considered as a valuable alternative to the end-to-end suture. The glass composition was not reported in the paper but, in a later work published by Jeans et al. [57], the authors commented on a previous study regarding a glass tube used for the repair of divided facial nerve in sheep and referred to the glass as Corglaes® (Giltech Ltd). In the Giltech Ltd website (www.giltech.biz), Corglaes® is described as belonging to the Na_2O-CaO-P_2O_5 system and can be designed with different compositions, dissolution rates and applications.

Some studies by Jeans et al. [57,58] and Starrit et al. [59] reported the use of a glass fibre wrap made of non-woven fibres of Corglaes® used for the treatment of divided median nerves in the upper forelimb or facial nerves of sheep (Fig. 4a). In one of these papers, the woven fibres were reported to be bonded with a biodegradable polymeric solution [57]. The wrap was designed to be porous to allow the passage of molecules (e.g. nutrients) towards the site of repair. The two nerve stumps were approximated to each other and glue or sutures were used to fix the wrap in a tubular form around the nerve stumps. The results showed nerve repair comparable to that obtained by microsurgical epineurial technique and by using wrap fixed with fibrin glue [57-59] or 6/0

polyglactin sutures [57]. Moreover, the wraps were completely resorbed after 7 months. Based on these findings, the authors stated that the use of the glass fibre wrap could be an effective alternative to microsurgical repair, since required less skill by the surgeon without the need for sophisticated microsurgical equipment.

The clinical use of NGCs is now effective for nerve defects up to about 3 cm [60] and thus the current scientific research is focused on the introduction of additional features and functionalities to the devices in order to improve their effectiveness for longer nerve defects. The main limiting factors for large nerve gaps seem to be the inadequate formation of fibrin cables inside the NGC, which is important to guide cells migration and axonal growth during regeneration, and an insufficient neurotrophic support [54]. One of the strategies that are currently investigated to improve the nerve regeneration into a NGC is the use of fibres of nanometric or micrometric diameter of different materials in order to support and guide the nerve tissue growth. In this context, fibres of different types of glass have been proposed to create a 3-D scaffold with an anisotropic structure, potentially carrying soluble factors, and able to sustain and direct the axonal regeneration. A first approach consists in using a bundle of fibres aligned inside a NGC lumen as reported by Bunting et al. [61], who showed that 45S5 Bioglass® fibres allowed the attachment and spreading of Schwann cells, which resulted to be aligned in longitudinal chains *in vitro*. These authors demonstrated that the presence of the fibres (length 0.5 cm, diameter 25 µm) inside a Silastic® conduit, used for the repair of a 0.5 cm gap in the sciatic nerve of adult rats, resulted in a re-innervation comparable to that of autograft treatment and ten times greater than the one obtained in empty conduits or unrepaired gaps. The hypothesized mechanism was the contact guidance of the regenerating axons and their associated non-neuronal cells.

Vitale-Brovarone et al. [62] studied the interaction of aligned resorbable fibres of TiO_2-containing phosphate glass (glass code $TiPS_{2.5}$, fibre diameters 25-80 µm) with glial cells (Neonatal Olfactory Bulb Ensheathing Cell line, NOBEC), and Dorsal Root Ganglia (DRG) neurons *in vitro* (Figs. 4b and c). It was reported that the phosphate glass fibres were permissive substrate for cell adhesion

and proliferation: on the aligned fibres, glial cells were well spread and enveloped the fibres while neurons showed bipolar morphology with neurites growing along the fibre axis direction and were longer than those observed on the control glass coverslip. Novajra et al. [63,64] developed resorbable hollow fibres of $TiPS_{2.5}$ phosphate glass which could be easily filled exploiting the capillary action with a liquid or a hydrogel solution containing a specific molecule (e.g. a growth factor), that could be released from the fibres with different kinetics depending on the filling material. An *in vitro* study showed that the fibre dissolution products did not show any negative effect on glial cells growth and pro-/anti-apoptotic proteins expression. A NGC was produced by placing a glass hollow fibre bundle into a poly(ε-caprolactone) (PCL) tube. The authors concluded that the filling of the fibres with a solution containing a specific growth factor would ideally impart to the hollow PCL/glass fibre system both topographical (i.e. anisotropic structures to direct cell growth) and trophic cues (e.g. neurotrophic support), which seem to be the key factors to improve the nerve regeneration for long nerve defects.

Another approach in the use of glass fibres to help in directing axonal regeneration is to assemble them within a natural polymer matrix without the use a tubular NGC. Marquardt et al. [65] produced fibrin scaffolds with embedded borate glass (13-93B3) fibres in a random (diameters 0.5-10 μm) or aligned configurations (diameters 50-200 μm). They found that neurite outgrowth of chick DRG neurons on the scaffolds in the presence of random fibres was comparable to that of the pure fibrin scaffold, while it proceeded in an oriented direction on the scaffold containing aligned glass fibres *in vitro*.

Kim et al. [66] produced 3-D scaffolds (length 3 mm) with aligned phosphate glass fibres (diameter 15 μm) embedded into a collagen matrix and used them for the repair of transected sciatic nerves of rats *in vivo* (Fig. 4d). This study extended the previous *in vitro* results reported by Vitale-Brovarone et al. [62] demonstrating that the presence of the fibres produced a faster axonal outgrowth in the initial regenerating period and promoted directional extension of axons if compared to pure collagen scaffolds *in vivo*.

The same collagen/phosphate glass fibre constructs were also studied for the treatment of spinal cord injury by Joo et al. [67]. Spinal cord injury can cause serious neurological deficits and compromises sensory and motor functions, as well as other related problems such as bladder and kidney infections, cardiac and respiratory dysfunctions and bowel diseases [68]. At present, the treatment consists in surgical intervention of stabilization and decompression of the spinal cord, drug therapy and rehabilitation, which can stimulate the spinal cord plasticity. Some devices for the treatment of spinal cord injuries are currently under investigation in the literature, including tubular guide and 3-D scaffolds of naturally derived and synthetic polymers; however, effective treatments or scaffolds are still to be found. Joo et al. [67] obtained promising results with collagen/phosphate glass fibre scaffolds *in vivo* (scaffold diameter 1.8 mm, length 3 mm) for the treatment of divided rat spinal cord. The presence of the fibres resulted in an improved locomotor and bladder function from 8 postoperative weeks compared to pure collagen scaffolds and some axonal growth from the proximal and distal stump was found only in the fibre-containing scaffold.

Micro- or nano-sized glass powder has also been recently proposed in combination with polymers to obtain composite devices for peripheral nerve regeneration. The presence of the glass in the composite material is considered to be beneficial for the improvement of the mechanical properties and also for the release of ions that can enhance the nerve healing process. Zhang et al. [69-72] investigated the use of glass powders (size < 45 μm) in the SiO_2-Na_2O-CaO-ZnO-CeO_2 system for the fabrication of composite NGCs with poly(lactic-*co*-glycolic acid) (PLGA) and Pluronic F127. The authors concluded that ion release from the glass in the composite material at appropriate concentrations may be advantageous for peripheral nerve regeneration, particularly due to the role of Ca^{2+} in the regulation of the nerve growth cone motility and the possible participation of Zn^{2+} in neurotransmission as well as its antibacterial effect [69,70]. This composite NGC showed analogous mechanical properties and even superior biocompatibility *in vitro* compared to the commercial PDLLA/caprolactone NGC (Neurolac®) [71,72].

Koudehi et al. [73] developed composite tubes of gelatin reinforced with nanopowders of SiO_2-CaO-P_2O_5-MgO glass (particle size 100 nm) and tested them *in vivo* for the treatment of 10 mm gap in the sciatic nerve of rats (Figs. 4e and f). The composite tubes resulted in the regeneration of the nerve, which after 3 postoperative months was comparable to a normal nerve in terms of functional and histological properties.

4. Skeletal muscle tissue engineering and ligament repair

After severe injury, such as volumetric muscle loss, the spontaneous regeneration process of skeletal muscle is not sufficient and the current surgical approaches show a limited success, thus requiring the investigation of new strategies and materials to sustain the tissue regeneration [74].

Few studies have been carried out with glasses for applications in muscle regeneration by the research group led by Prof. Jonathan Knowles. Ahmed et al. [75] found that some fibres of phosphate glass in the CaO-Na_2O-Fe_2O_3-P_2O_5 system allowed attachment, proliferation and differentiation of conditionally immortal muscle precursor cell line with the formation of myotubes along the axis of the fibres. Shah et al. [76] found that human masseter-derived cells seeded on a 3-D mesh construct not only attached and proliferated but also migrated along the fibres forming multinucleated myotubes. It was also found that 3-D aligned fibre scaffolds were able to support unidirectional cell alignment and caused an up-regulation of genes encoding for myogenic regulatory factors [77], even when the glass fibres were embedded into a collagen gel to form a composite scaffold [78].

The same research group also studied the suitability of phosphate glass (CaO-Na_2O-Fe_2O_3-P_2O_5 system) fibres bundles for the repair of bone-ligament interface by assessing the fibre solubility as well as the growth and functional gene expression of human cells (primary osteoblasts and fibroblasts) seeded onto the scaffolds and maintained in culture for up to 21 days [79]. Glass dissolution rate was crucial in determining the nature of cell-glass composition interaction as the

most soluble fibres (containing 1 mol.% of Fe_2O_3) failed to support the survival of cells beyond 7 days in culture, whereas substantial growth rate took place on the least soluble fibres with 3 mol.% of Fe_2O_3. The authors suggested that these resorbable scaffolds could be used to accommodate the separate seeding of two cell populations in a co-culture arrangement, in order to potentially simulate *in vitro* the anatomical structure of a bone-ligament tissue interface.

5. Ocular implants

The importance of glass in ophthalmology is well known since ancient times as it is has been used for centuries to make external lenses to correct visual deficiencies. Maybe, it is less known that the first potentially implantable device comprising a glass element was still developed in the context of ocular surgery: in the late 1700s, Pellier de Quengsy suggested the use of a thin silver-rimmed convex glass disc as a keratoprosthesis (artificial cornea) and described in great detail the surgical instruments suitable for its implantation [80]. However, there is no evidence that this device was ever implanted and only in the mid 1800s a glass keratoprosthesis was actually inserted in rabbit and human eyes [81]. Since then, the use of glass as optical element in artificial corneas was repeatedly proposed with controversial results and, soon after the Second World War (WWII), the lighter and more resistant poly(methylmethacrylate) (PMMA) began to be adopted as preferable material.

Historically, glass was also used for aesthetic purposes in the fabrication of artificial eyes that roughly filled the orbital socket after removal of the ocular globe. In the late 1800s Mules first described in detail the surgical placement of a hollow glass sphere into the orbital cavity of a human patient [82] and, in the early 1900s, orbital implants (to be inserted in the anophthalmic socket) began to be coupled with external ocular prostheses (to be placed between the closed conjunctival surface covering the orbital implant and the eyelids) to improve the aesthetic appearance of the patient's face. Glass eyes, however, had to be worn with caution as they were brittle, prone to

implosion with acute changes in temperature and susceptible to etching from exposure to body secretions; after the WWII, PMMA orbital implants and ocular prostheses were introduced to overcome glass shortcomings and, in recent years, implantation of glass spheres has still been performed only in selected cases [83,84].

In the above-mentioned applications, the (implantable) glass element was intended to perform an optical or aesthetic function; on the contrary, the major added value carried by the use of bioactive glasses in ophthalmic surgery is the improvement of implant biointegration[2], which is crucial for the postoperative success of the ocular device.

5.1. Artificial cornea

Being the silicate bioactive glasses hydrophilic, they have been proposed in a few studies for the manufacturing of anchorage elements, commonly referred to as "skirts", around the optical core of keratoprostheses with the aim of encouraging biocolonization by corneal cells and thereby *in situ* fixation of the implant[3]. The first formulations to be investigated in the late 1970s belonged to the group of Ceravital® glass-ceramics that, however, tended to progressively dissolve after contact with biological fluids *in vivo* and, therefore, were considered unsuitable for safe prosthetic anchorage [85-88]. In the early 1990s, the intracorneal biocompatibility of Bioverit® I (glass-ceramic in the SiO_2-MgO-CaO-Na_2O-K_2O-Al_2O_3-F-P_2O_5 system, with fluorophlogopite mica and apatite as crystalline phases) and Bioverit® II (glass-ceramic in the SiO_2-MgO-CaO-Na_2O-K_2O-Al_2O_3-F-P_2O_5-TiO_2 system with tetrasilicic mica and apatite as crystalline phases) was investigated in rabbit eyes; in spite of the good results achieved (the materials were incorporated into the host

[2] In the context of ocular surgery and referring to the two main application fields of bioactive glasses, the term "biointegration" denotes, distinctively, the fibrovascularization of porous orbital implants (i.e. the in-growth of viable vascular connective tissue which helps to hold the implant in place and to discourage bacterial colonization) and the anchorage of keratoprosthesis to the host corneal tissue.

[3] The skirts around the currently-used keratoprostheses are made of inert polymers (e.g. poly(2-hydroxyethyl methacrylate) hydrogel in the AlphaCor device) or, in the case of the osteo-odonto-keratoprostheses, comprise autologous tissues from patient's tooth and buccal mucosa. In the latter case, harvesting of autologous tissues requires extra-surgery and is stressful to the patient.

corneal tissue without immune reactions and irritations), the investigations were discontinued [89]. Few years later, a group of Finnish researchers deposited apatite/wollastonite (A/W) glass-ceramic coatings on the titanium skirt of keratoprostheses implanted in rabbits (Fig. 5a) in the attempt to avoid the in-growth of corneal or conjunctival epithelium into the anterior chamber of the eye, which may lead to infection and extrusion of the implant as well as to the development of retroprosthetic membrane and secondary glaucoma [90]. The results were encouraging and the A/W coatings were found suitable for the intended aim fastening the prosthesis to the corneal tissue before the epithelium grows inward, but also in this case the studies were discontinued probably due to the advent of polymeric porous keratoprosthetic skirts, that exhibited more appropriate physico-mechanical properties for use in the ocular environment.

More recently, Santos et al. [91] investigated the properties of a phosphate glass ($65P_2O_5$-15CaO-$10CaF_2$-$10Na_2O$ mol.%)-reinforced HA porous composite (porosity up to 50 vol.% using PVA as a pore former) for potential use as a keratoprosthetic skirt. Degradation studies showed that no mass loss was found under simulated physiologic conditions (immersion in Tris solution); on the contrary, a significant mass loss was observed under acidic conditions (immersion in citric acid solution) that mimicked an environment colonized by bacteria. The biological performance of these phosphate glass/HA composites was satisfactory when cultured with human corneal fibroblasts, that invaded the material porous network, grew and proliferate over a 14-day culture period. The mean pore size of 110 μm was found adequate to allow the implant to be colonized by corneal cells.

Laattala et al. [92] proposed composite keratoprosthetic skirts (Fig. 5b) in which PMMA was mixed with a significant amount (40 wt.%) of bioactive glass particles (45S5 Bioglass®, S53P4, 1-98 ($53SiO_2$-$6Na_2O$-22CaO-$2P_2O_5$-$11K_2O$-5MgO-$1B_2O_3$ wt.%) and FL107 ($64SiO_2$-$10Na_2O$-16CaO-$2P_2O_5$-6MgO-$2B_2O_3$ wt.%)) with the aim to promote corneal cell adhesion (which hardly occurs if PMMA alone is used). A decrease of compressive strength and elastic modulus after soaking in simulated aqueous humour was observed due to bioactive glass dissolution with an associated porosity increase; this behaviour was partially suppressed by the formation of an apatite layer on the

glass particle surface. As a short remark to this study, it is worth underlining that the progressive glass dissolution and the weak interfaces between glass particles and PMMA matrix (due to the lack of a covalent bond) are crucial concerns since the penetration of water molecules into the composite could eventually lead to local disintegration of the skirt and loosening of the keratoprosthesis *in vivo*.

Huhtinen et al. [93] fabricated porous skirts using two melt-derived experimental silico-boro-phosphate glasses (1-98 ($5.9Na_2O$-$7.1K_2O$-$7.6MgO$-$23.9CaO$-$0.9B_2O_3$-$0.9P_2O_5$-$53.8SiO_2$ mol.%) and 28-04 ($4.9Na_2O$-$7.2K_2O$-$9.0MgO$-$16.2CaO$-$2.6B_2O_3$-$60.1SiO_2$ mol.%)) that, after being reduced in powder, were pressed and finally sintered to produce ring-shaped structures with interconnected porosity. *In vitro* tests with human keratocytes showed that none of the porous bioactive glass structures induced a cytokine-driven inflammatory response and the adherent keratocytes exhibited a typical elongated, spindle-shaped morphology which suggested a good adhesive potential.

An interesting *in vivo* investigation was reported by Liang et al. [94], who implanted experimental glass-ceramic disks (diameter 8 mm, thickness 0.5 mm, pore diameter 20-70 μm, porosity 37-62 vol.%) in albino rabbit corneas. The implants with porosity above 50 vol.% were all extruded due to breakage and some clinical complications (corneal oedema with severe degrees of corneal neovascularization, opacity of the corneal lamella) were observed in the other cases. The chosen glass-ceramics were judged unsuitable as materials for keratoprostheses because of excessive roughness, thickness and brittleness; perhaps, an optimization of the structural design parameters (e.g. implant size, porosity and interconnectivity) maintaining unaltered the material formulation might lead to more satisfactory results in future studies.

As a general comment, it is worth underlining that bioactive glasses for keratoprosthetic skirts should be primarily selected on the basis of their chemical/biological stability, as even a moderate dissolution of the glass over time may destabilise the prosthesis. A soluble glass could be an option only if incorporated in a stable backbone structure which is essential to maintain the optical core in

the correct position, as suggested in the study by Santos et al. [91]. In these cases, accurate investigations are necessary to explore the effects of the ion dissolution products from bioactive glasses in the ocular environment as well as the associated pH change.

5.2. Orbital implants

Commercial porous orbital implants used in current enucleation/evisceration procedures[4] are made of hydroxyapatite (HA), polyethylene (PE) or alumina [95]; in the last 20 years, however, bioactive glasses have also been investigated as alternative materials by a few groups of researchers worldwide.

In the late 1990s, Xu and co-workers [96] implanted bioactive glass-ceramic porous orbital implants in enucleated rabbits and observed no rejection over a 6-month postoperative follow-up; ultrasound examination revealed a venous-flow-like spectra in the implants after 3 months and histological analysis showed that around 90% of the implant pores were filled by fibrovascular tissue after 6 months from implantation. Encouraged by these promising results, the same authors experimented glass-ceramic porous orbital devices in 102 human patients, declaring a success rate of 96.1% after a 2-year follow-up [97].

More recently, the use of bioactive glass to fabricate orbital implants was claimed in a recent patent by Richter et al. [98], but no manufacturing or clinical studies have been reported yet in the literature on this type of implant.

Milani Brandao et al. [99] implanted 45S5 Bioglass® and Biosilicate®-derived glass-ceramic cones (Fig. 6a) in 45 eviscerated rabbits, that were then sacrificed at 7, 90 and 180 days after surgery for histo-morphological examination. Biosilicate® is a patented bioactive glass (SiO_2-CaO-Na_2O-P_2O_5 quaternary system) originally developed by the research group of Peitl and Zanotto in Brazil [100].

[4] Evisceration involves the removal of the contents of an eyeball, with the sclera and muscle attachments left intact; enucleation is more radical and involves the removal of the entire globe from the orbital socket, together with the scleral envelope and a portion of the optic nerve, while the conjunctiva, Tenon's capsule and extraocular muscles are usually spared. The latter procedure is highly recommended in the case of ocular tumours.

Two types of Biosilicate®-derived glass-ceramic cones were implanted in rabbits [99] depending on the applied thermal treatment: the heat treatment produced a calcium-sodium silicate as the crystalline phase of Biosilicate® I, while the nucleation of apatite was induced in Biosilicate® II. No cone extrusions from the ocular socket were reported; histological examinations revealed the formation of a pseudocapsule around the cones with an inflammatory reaction that progressively decreased over time. 45S5 Bioglass® and Biosilicate® I conical implants caused a milder inflammatory reaction and the formation of a thinner fibrous capsule with respect to Biosilicate® II ones; further studies will contribute to better assess the suitability of these two materials as orbital implants and their possible added values compared to existing solutions.

Another interesting application involves the use of bioactive glass as a "contingency plan" to fill old peg[5] tracts and to permit re-pegging in porous HA orbital implants, if the initial drilled tunnel was not perpendicular and central to the implant surface [101]. This approach has been reported in a study on 3 patients who had pegged HA orbital implants with related complications and, over a 2-year period, did not respond to conservative treatment. After removal of the old peg, the hole was partially filled with bioactive glass and, after 2 months, 2 patients underwent successful implant re-drilling followed by insertion of a new titanium peg, with satisfactory connection to the ocular prosthesis and absence of complications over a 3-year follow-up.

In a couple of recent studies, bioactive glass-coated PE porous spheres were experimentally implanted in enucleated rabbits [102] and human patients [103] to investigate the effect of bioactive glass on the fibrovascular in-growth within the implant pore network. Interestingly, however, the inclusion of bioactive glass particulate did not seem to significantly promote the rate of fibrovascularization, and probably this was the reason why the investigations on such composite implants were (apparently) discontinued.

[5] "Pegging" is a surgical procedure that can be optionally performed after some months from orbital implant placement in the anophthalmic socket (primary surgery). In this procedure, a hole is drilled into the front surface of the implant and a polymeric or metal peg is inserted into this hole. The peg articulates with a cavity in the back surface of the aesthetic ocular prosthesis, thereby providing improved motility and a more "life-like" appearance to the prosthetic eye.

Very recently, Ye et al. [104] coated macroporous HA orbital implants with a thin layer of CuO-containing MBG (Figs. 6b and c): the aim of this research was to combine the antibacterial effect of released Cu^{2+} ions with the drug uptake/release capacity of the mesoporous coating. Specifically, Cu-MBG coatings with 0-5 mol.% of CuO were prepared by direct dipping of the HA porous implant into the Cu-MBG sol precursor, followed by evaporation, ageing and calcination. With the peculiarity of releasing antibacterial Cu^{2+} ions as the glass degrades (viability of *Staphylococcus aureus* and *Escherichia coli* was inhibited) and good drug uptake/delivery ability (in this study ofloxacin), Cu-MBG coating could be a promising, multifunctional tool in the prevention of implant-related infections. Furthermore, there is an additional, perhaps even more important reason why this approach is so fascinating – which however was not highlighted in that research article: several tissue engineering studies have demonstrated that Cu^{2+} ions released from bioactive glasses induce migration and proliferation of endothelial cells during *in vitro* culture (angiogenic effect) [105,106], which could promote and accelerate fibrovascularization of porous orbital implants with significant increment of the postoperative success rate.

It is worth underlining that soluble glasses can be used only in form of a coating on a non-absorbable structure which acts as a permanent "skeleton" of the orbital implant, in order to ensure socket volume replacement for the patient's lifetime without the need for subsequent surgical substitution.

5.3. Artificial retina

In spite of the enormous efforts and advances in clinical treatment of eye diseases, there is no established method to prevent or cure some degenerative processes in eye, such as age-related macular degeneration and retinitis pigmentosa. Artificial retina (also termed "bionic eye") aims to substitute degenerated visual photoreceptors and neural structures for stimulating intact neural tissue so that a meaningful visual sensation is obtained. Subretinal implants involve the placement

of a Si-based microphotodiode array (MPDA) in the subretinal space: an external camera connected to a pair of glasses worn by the patient transmits wirelessly the electromagnetic signals to the implanted device, and a photovoltaic charge is then generated in each photodiode cell and transferred to the adjacent microelectrode for stimulation of the bipolar cells. In the late 1990s, a group of German researchers manufactured a MPDA on a silicon wafer using complementary metal-oxide semiconductor process technology [107]; after completion of electrically active structures, a 500-nm thick passivation layer consisting of pure SiO_2 glass was applied using tetraethylorthosilicate (TEOS) as a precursor. *In vivo* animal experiments (rabbits and pigs) revealed a decay of the SiO_2 passivation layer and pit corrosion of the underlying silicon for implantation periods above 6 months. In order to overcome these shortcomings, other materials have been experimented later to produce the stimulation microelectrodes (e.g. nanoporous TiN) as well as the passivation layer (polyimide, bencocyclobutene and parylene C) with better outcomes [108]. The subretinal implant Argus II (60 electrodes) has recently received approval for commercial use in Europe (2011) and USA (2013) [109]. Although the choice of pure SiO_2 glass as a protective layer was not the most fortunate, it is an interesting example about the use of a biocompatible glass as a thin passivation film in a bionic device (subretinal implant); in the light of the latest advances in tissue engineering and bioactive glass technology, maybe in the future the concept of using glass in artificial retinas could be resurrected to develop new, currently unexpected applications also in this research field.

6. Treatment of ear diseases

The double ability of a subset of bioactive glasses and glass-ceramics to bond both to bone and to soft connective tissues has been exploited in the development of artificial middle ear small bones (ossicles) and cochlear implants for ear surgery. Both uses have been traditionally classified as special osseous applications of bioactive glasses; however, they are mentioned in the present review

as the function of the material is not limited to bone-bonding ability and addresses the functional recovery of a complex sense organ (the ear).

In the late 1970s, glass-ceramics belonging to the Ceravital® group were introduced in middle ear reconstructive surgery to replace the small auditory ossicles damaged by chronic infection [110], which leads to problems in sound conduction from the tympanic membrane to the cochlea. Animal studies demonstrated that this material was well-tolerated by surrounding tissues, without inducing irritation or negatively affecting cochlear functions [111]. Zikk et al. [112] implanted Ceravital® granules in the middle ear of guinea pigs (Fig. 7a) and reported a temporary hearing loss in the early postoperative period, which solved spontaneously around the 20th postoperative day when the auditory response returned to pre-surgical levels. This problem was purely conductive and due to surgical manipulation of the middle ear as well as to Ceravital®-induced biochemical reaction, which interfered temporarily with the middle ear conductive system (formation of a surface silica gel layer followed by collagen fibres formation and tight interfacial bonding between the granules and the surgical bed). Generally good long-term outcomes of Ceravital® implants were observed in humans over a 8-year follow-up, although some concerns about partial resorption of the implant over time still linger on [113].

In 1985, cast 45S5 Bioglass® structures (roughly similar to truncated cones) were clinically approved for ear ossicles replacement. This implant, called "Bioglass® Ossicular Reconstruction Prosthesis", was the first bioactive glass device cleared for marketing under the commercial name "MEP®" [114-116]. It is interesting to underline that survivability of MEP® is considerably longer compared to that of "traditional" alumina implants used for the same purpose, since the latters do not bond to the eardrum and, therefore, gradually erode through it and are extruded within 2-3 years [117]. On the contrary, Bioglass® implant forms a tight bond both with the collagen fibres of the tympanic membrane of the eardrum and with the remaining bone of the stapes footplate and, thereby, is anchored on both ends, which prevents extrusion. Sound conduction is generally excellent without excessive fibrous tissue growth to impair sound transmission; however, some

issues exist about the long-term fate of such implants. Rust et al. [118] reported that no micro-movements at the Bioglass® implant-tissue interface occurred, thus the implant remained safely anchored in the correct position over a maximum follow-up of 126 months. On the contrary, Bahamad and Merchant [119] observed that Bioglass® ossicular implants tended to break down into small fragments and to be partially resorbed by a host response within the middle ear after 14 years from implantation (Fig. 7b). Therefore, these results warrant caution in the use of ossicular prostheses made of Bioglass® (and Ceravital®, too) and further long-term investigations would be highly desirable to achieve more definite conclusions.

In the context of ossicular replacement, it was also proposed the use of glass ionomer cement, that is produced by an exothermic reaction between a CaO-Al_2O_3-SiO_2-CaF_2 glass and a polyalkenoic acid solution. In both animal and clinical studies, the implants were coated with a mucosal layer within a short time and were assessed as bio-stable and well-tolerated in the middle ear environment [120,121]. However, this material has been fallen in disuse for middle ear surgery due to some concerns about aluminium lethal intoxication after contact with brain liquor [122].

At present, the most commonly adopted solutions for ossicle replacement still remain the use of autografts, inert polymers or HA/PE composite (Hapex®) implants [119]. A promise for the future might come from the use of Biosilicate®, that was recently assessed to be safe (in powder form) in the middle ear environment of guinea pigs (absence of signs of oto- and vestibular toxicity at 90 days) [123]. Recently, some authors also pointed out the potential of Bioverit® II that, after implantation in the middle ear, tends to be coated with an epithelial layer exhibiting absence of inflammation but also minimal osteogenic response [124]; antibacterial effect against gram-negative bacteria was also reported as an added value [125]. In order to improve the bone-bonding ability of Bioverit® II, it was proposed the coating of the implant with a nanostructured silica layer and promising results in animal middle ear models (mice and rabbits) were reported [126,127].

The second application of bioactive glasses for ear surgery concerns the development of cochlear implants for those people who are profoundly deaf due to damage to sensory hair cells in their

cochleas. In these patients, the implants often can enable sufficient hearing for better understanding of speech; although the quality of sound is different from natural hearing, with less sound information being received and processed by the brain (typically the sounds are perceived at much lower frequencies), many patients are able to hear and understand speech as well as environmental sounds. Essentially, a cochlear implant comprises a microphone, a speech processor that selectively filters sound to prioritize audible speech and a transmitter on the external part of the ear, followed by a receiver secured in the bone under the skin that converts the signals into electric impulses and sends them to an array of electrodes placed through the cochlea; finally, the impulses are sent to the brain through the auditory nerve system [128]. One of the first prototypes, developed in the 1980s at the University College London [129], was based on an array of four platinum electrodes insulated by medical-grade Al_2O_3 and anchored in the bone with a 45S5 Bioglass® sleeve. The Al_2O_3 envelope provided mechanical and dielectric stability to the device, while the 45S5 Bioglass® connector bonded both to the bone and to the soft connective tissues as it protruded through the skin. This hermetic, percutaneous seal – from which the common name Bioglass®-EPI (extracochlear percutaneous implant) – provided mechanical stability and prevented infection from electrodes migration, thereby protecting the patient who had to unplug the electronics at night. In a recent version of the device, the design concept was essentially maintained unaltered but fixation to bone occurred by a titanium pedestal; 45S5 Bioglass® was no longer used due to some concerns about the maintenance of adequate mechanical integrity over time (Bioglass® is an intrinsically brittle material and, furthermore, undergoes moderate dissolution in the biological environment) [130].

7. Treatment of liver cancer and applications of radioactive glasses

In situ irradiation of cancer-affected organs has a number of advantages in comparison to using external radiation sources, since the radiation is applied to a more localized area, higher doses can

be applied for shorter periods with lower patient's discomfort and there is less damage to healthy tissue [131]. An interesting method for internal therapeutic irradiation involves the creation of an appropriate radioisotope by neutron bombardment in biocompatible glass beads that will be subsequently injected into the patient's blood flow [132]. These microspheres must be insoluble in the biological fluids to avoid dissolution and migration of the radioactive material via the blood circulation into the body, and should be sized to lodge in the capillary bed of the organ to be treated. A further criterion for material selection involves the absence of unwanted elements that could become radioactive upon neutron bombardment.

For the treatment of liver cancer, Day and co-workers first proposed the use of injectable Y_2O_3-Al_2O_3-SiO_2 glass microspheres (diameter of 25 μm) with as much as 50 wt.% yttrium oxide, characterized by high durability due to the absence of alkali ions [132-134]. Before arterial infusion, the glass beads were bombarded by neutrons that create ^{90}Y, a radioisotope that is a short-half-life (64 h) and short-range β-rays emitter. In this way, a localized dosage of up to 15000 rad could be delivered, whereas a maximum of 3000 rad under external radiation can be tolerated by the patient. At present, radioactive glass microspheres (marketed under the commercial name of TheraSphere®) are clinically used for the treatment of unresectable hepatocellular carcinoma, metastatic liver cancer and cholangiocarcinoma at a number of clinical centres in EU countries, Canada and USA after receiving FDA approval in 1999. This therapy leads to a significant improvement of survival times and quality of life for the patients.

A variation of this approach involves the incorporation of a radionuclide in protective insoluble microcapsules that will be injected into the patient's body and subsequently removed; an example is the treatment of prostate cancer using ^{142}Pr glass seeds [135].

In situ radiotherapy using Dy_2O_3-Li_2O-B_2O_3-Al_2O_3 glass seeds, that are neutron activated to form ^{165}Dy, has also been proposed recently for the treatment of arthritic joints [136]; in this case, injectable glass beads must be soluble in biological fluids as their long-term persistence in the synovial fluid would cause disease aggravation.

8. Embolization of uterine fibroids

In recent years, embolization of vascular tumours has gained increasing attention as an important tool in minimally invasive surgical intervention; a typical field of application is the treatment of symptomatic uterine fibroids, which are benign tumours from smooth muscle tissue (leiomyomas) that affect about 25% of all women [137,138]. Uterine fibroid embolization (UFE) comprises the intentional occlusion of blood vessels with embolic materials so that blood flow is obstructed, thereby leading to infarction of leiomyomas and reduction of fibroid-associated pain. UFE is a safe, effective procedure and provides similar results to hysterectomy, but has the advantage of shorter recovery times, uterine preservation and maintenance of potential for future pregnancy over conventional surgical intervention. An ideal material for UFE should be radiopaque, which is not fulfilled by polymeric microspheres that are used in the current surgical practice. In order to achieve this requirement, Kehoe et al. [139] recently demonstrated the potential suitability of various types of glass microspheres (size within 45-212 μm) belonging to the SiO_2-CaO-ZnO-La_2O_3-TiO_2-MgO-SrO-Na_2O system. The same research group developed an interesting set of models to analytically predict the composition-density and composition-cytocompatibility relationships for these experimental materials, that exhibited an *in vitro* biocompatibility with fibroblasts generally equivalent or enhanced in comparison to commercially available embolic polymeric agents [140]. Ion release profiles of glass microspheres were also determined and no genotoxic effect was observed in the bacterial mutation Ames assay, which further supports the potential suitability of these materials as embolic agents [141].

9. Summary and outlook

Only 45 years ago, before 45S5 Bioglass® was invented by Prof. Larry Hench and co-workers [2], the concept of a material that could bond to living tissues with no rejection or long-term interfacial problems seemed impossible. Since then, bioactive glasses transformed biomedical science and technology and the research still continues today with impressive results: none of the latest applications were forecast when the research began, evolving over the years beyond orthopaedic and dental implants towards new, smart applications in soft tissue engineering and organ regeneration [19].

Bioactive glasses are characterized by a great versatility from the viewpoints of composition and manufacturing, on which researchers can act to develop ever more suitable biomaterials and implants for tissue repair and targeted therapy. Small changes in the bioactive glass composition can lead to very different physico-chemical, mechanical and biological properties (e.g. mechanical strength, surface reactivity, dissolution kinetics), that could be therefore properly designed and modulated depending on the implantation site as well as on the specific implant scope.

In this regard, a few short remarks should be dedicated to better clarify the meaning of the term "bioactive", that is commonly used to define a special set of implantable glasses. In biomaterials science and regenerative medicine, the term "bioactivity" refers to the ability of a biomaterial to perform a specific function required to generate the most appropriate beneficial cellular or tissue response in a specific situation. Of course, bioactivity implies biocompatibility, i.e. the ability of a biomaterial to perform its function without eliciting any undesirable local or systemic effect in the recipient [142]. Initially, bioactivity referred to materials that could bond to bone (e.g. HA), but 45S5 Bioglass® was found to bond also to soft tissues [14] and to stimulate new bone growth through the release of Si and Ca ions having a genetic effect on cell functions [143]. Furthermore, the researches carried out over the last 30 years have demonstrated that also other ions released from new types of glass, the shape itself of the implant (e.g. micro-/nano-sized glass fibres) or intrinsic atomic properties of the material (e.g. radioactivity) are all factors that can be exploited to induce a particular cell or tissue response in a broad range of applications, not restricted to bone-

bonding ability. Therefore, the word “bioactive” widely expanded its meaning and should be always contextualized depending on the specific application, as summarized in Table 2.

Many studies have demonstrated that a powerful strategy to design glass-derived products able to elicit a desired biological response involves the introduction of therapeutic ions into the bioactive glass formulation; the subsequent release of these ions after exposure to a physiological environment could allow an antibacterial, anti-inflammatory or angiogenic effect to be obtained [10,105,143]. Antimicrobial properties are particularly crucial if infections are one of the primary cause of implant failure (for instance in wound dressing and ocular implants) and, in general, in all cases where cells have to compete with bacteria to colonize the implant. Angiogenesis is crucial for the development of regenerated tissue and could be particularly desirable in the case of wound healing [43,44] and porous orbital implants, in which fibrovascularization is essential to hold the device in place and to discourage bacterial colonization [94,144].

The introduction of metal ions is usually economical and compatible with the typical processes used for bioactive glass production (e.g. high-temperature melting, sol-gel method, sintering), which on the contrary are often incompatible with the incorporation and stability of organic moieties and drugs that might be used for the same purpose. Metal oxides such as CuO, Ag_2O and Fe_2O_3, apart from exerting an antibacterial effect via the release of the associated metal ions, can also modulate the glass solubility [9,13,79]. The use of soluble materials, however, deserves careful attention depending on the context of application. If used for tissue engineering purposes (e.g. for muscle or nerve repair), bioactive glass dissolution kinetics must be compatible with the healing rate of regenerated tissue; on the contrary, glasses with high chemical and biological stability should be selected to produce orbital implants and keratoprosthetic skirts, that must remain *in situ* indefinitely during the patient’s whole life to ensure an adequate socket volume replacement without undergoing degradation. Long-term integrity issues related to Bioglass® ossicular implants were also highlighted by Bahmad and Merchant [119], who suggested caution in using this material for such an application. Partial resorption over time of the Bioglass® sleeve that fixed the cochlear

implant to temporal bone led to its substitution with a titanium pedestal in the most recent versions of the device [130]. As a general indication, bioactive glasses and glass-ceramics with high chemical and biological stability have to be developed if their function is the safe anchorage of an implantable prosthetic device that must not migrate over time [145,146]. Insoluble glasses are also highly desirable to produce injectable radioactive microspheres for cancer treatment [134].

Incorporation of a radiopaque phase, such as zirconia (ZrO_2), in the glass formulation would allow better visualization of the implant under radiographic imaging and detection of problems associated to undesired postoperative migration [147]. Furthermore, ZrO_2 could be a valuable alternative to the potentially toxic La_2O_3 [140] as a radiopaque agent in glass microspheres for uterine fibroid embolization.

Bioactive glasses are attractive also from a technological viewpoint due to the relative ease of processing associated to their production. In the context of hard tissue repair, bioactive glasses and glass-ceramics are usually synthesized in the form of powder to finally produce 3-D porous scaffolds with different size, shapes, pore architecture and mechanical properties through a number of relatively easy, versatile methods such as sponge replica technique [148], sol-gel foaming [149], polymeric particles burning-out [150], gel-cast foaming [151] and rapid prototyping techniques [152]. In non-osseous applications, where adequate compliance with soft tissues is required, bioactive glasses are often introduced as inorganic fillers in a polymeric matrix to increase the mechanical properties (e.g. composite nerve guidance channels [70-73]). Other uses concern the production of coatings to modulate cell adhesion (e.g. titanium keratoprosthesis coated with A/W glass-ceramics [90]) and to impart special properties, such as an antibacterial effect (e.g. glass-coated polymeric sutures [28] or Ag-doped phosphate glass/polymer composites in the form of film or topical powder for wound dressing). If glasses are processed in the form of mesoporous materials, they can also easily uptake specific molecules, such as anti-inflammatory drugs, to be released *in situ* postoperatively to elicit an appropriate therapeutic effect [51,104]. Incorporation of MBGs in soft matrices, such as injectable pastes or polymer patches, could be a valuable tool for

the local release of chemotherapic and antineoplastic drugs for cancer treatment [153]. Phosphate glasses, thanks to their low glass transition temperature, can be drawn in the form of dense, hollow and/or mesoporous fibres, which are very attractive for the fabrication of muscle and nerve tissue engineering constructs.

Looking at the future, the development of biofabrication strategies involving the use of bioactive glasses could open new doors in the field of organ printing. Biofabrication can be defined as the production of complex biological products (including portions of implantable tissues) from raw materials such as living cells, molecules, extracellular matrices and biomaterials [154,155]. Biomaterials science, cell/developmental biology and mechanical engineering are the main disciplines contributing to this emerging technology, the use of which is still mainly limited – probably due to technological issues – to "soft materials" (polymer-cells-biomolecules constructs) without the incorporation of a bioceramic phase.

Due to the need for adequate compliance with soft tissues and organs, biofabrication could be a valuable tool for the tailoring of smart composites comprising a soft polymeric matrix and a bioactive glass phase (Fig. 8) carrying special added values (e.g. improvement of the mechanical properties, antibacterial effect, angiogenesis stimulation or local drug delivery). Furthermore, biofabrication could pave the way for the simultaneous regeneration of multiple tissues by using a functionally graded construct which comprises different cell types, biomaterials and architectural/topological features. In this regard it is instructive to cite a recent work by Liverani et al. [156], who developed a relatively simple strategy for the production of 3-layer stratified scaffolds with potential application in osteochondral tissue engineering by integrating sponge replication, freeze-drying and electrospinning. 45S5 Bioglass® was used for the fabrication of the rigid bioactive substrate intended to be in contact with bone tissue, chitosan and alginate solutions were used to build by freeze gelation the interface between the porous glass layer and the soft cartilage side of the construct, and a chitosan-based electrospun nanofibrous membrane was selected for the upper layer of the scaffold. In this design, the intermediate layer has multiple

functions providing adherence between the other scaffold components and preventing delamination. This study could represent the starting point for the development of advanced multifunctional and "multi-tissue" engineering scaffolds, with the aim to concretize a not so remote dream: if today, describing the regenerative properties of bioactive glasses, we can rightly state "not only for bone and teeth" (as reviewed in this article), we do hope that, in the next few years, it will be possible to say "not only for one tissue at the same time".

Acknowledgements

The research leading to these results has received funding from the EU Seventh Framework Programme (FP7/2007-2013) under grant agreements no. 286548 (MATCh), no. 280575 (Restoration) and no. 264526 (GlaCERCo).

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Figure legends

Fig. 1. Chronology of the special applications of bioactive glasses outside the skeletal system.

Fig. 2. Antibacterial sutures: Mersilk® suture (a) before and (b) after being coated with Ag-doped bioactive glass particles (images adapted from Blaker et al. [29] © Wiley Periodicals).

Fig. 3. Ultrathin hollow MBG fibres: (a) as produced fibre; (b) fibre surface after soaking for 8 h in SBF (small spherical apatite structures, supposed to have a haemostatic effect, are clearly visible) (images adapted from Hong et al. [51] © Wiley-VCH).

Fig. 4. Examples of the use of bioactive glasses for peripheral nerve regeneration: (a) wrap of non-woven mesh of phosphate glass fibres bonded with a biodegradable polymeric solution used to treat the divided median nerve in the upper forelimb of sheep (image adapted from Jeans et al. [57] © British Association of Plastic, Reconstructive and Aesthetic Surgeons); (b) glial cells enveloping the glass fibres and (c) dorsal root ganglia neurons showing long neurites extended along the fibre axis direction (images adapted from Vitale-Brovarone et al. [62] © Elsevier); (d) SEM micrograph showing a section of a phosphate glass fibre/collagen 3-D scaffold for peripheral nerve repair (image adapted by Kim et al. [66] © Wiley); (e) and (f) composite tubes (internal diameter 1.6 mm) of gelatin/SiO_2-CaO-P_2O_5-MgO glass nanopowders (size 100 nm) tested for the treatment of 10 mm gap in the sciatic nerve of rats (image adapted from Koudehi et al. [73] © Springer).

Fig. 5. Bioactive glasses and glass-ceramics for artificial cornea: (a) histological picture of rabbit eye with a keratoprosthesis supported by a titanium flange coated with A/W glass-ceramic (main image: the small arrows point to the intact aspect of the cornea under the optical element “O”, whereas the large arrow points to the hole in the left half of the supporting flange; inset: there is a

tight contact between the A/W glass-ceramic coating and the corneal matrix tissue, while the corneal epithelium "e" shows no in-growth but has attached to the bioactive glass-ceramic (bgc) coating on the part supporting the optical element) (images adapted from Linnola et al. [90] © Academic Press Limited); (b) PMMA/bioactive glass composite skirt around the optical PMMA cylinder in an experimental keratoprosthesis (images adapted from Laattala et al. [92] © Elsevier).

Fig. 6. Bioactive glasses and glass-ceramics for orbital socket surgery: (a) conical orbital implants (anterior diameter 10 mm, posterior diameter 3 mm, length 12 mm) made of 45S5 Bioglass® (left), Biosilicate® I glass-ceramic (centre) and Biosilicate® II glass-ceramic (right) (image adapted from Milani Brandao et al. [99]); (b,c) MBG-coated porous HA scaffolds for possible use as orbital implants: on the left, photograph showing the outward appearance of the HA devices with different MBG coating (containing 0, 2 and 5 mol.% of CuO), on the right, morphology of porous HA coated with 5 mol.% Cu-doped MBG (images adapted from Ye et al [104] © Springer).

Fig. 7. Bioactive glasses and glass-ceramics for ossicular replacement: (a) Ceravital® granules with size in the 0.3-0.7 mm range (C) in the middle ear of a guinea pig (J = incudo-stapedial joint, W = round window) (image adapted from Zikk al. [112] © Springer-Verlag); (b) 45S5 Bioglass® prosthesis implanted for 14 years: the implant has become fragmented, host response consists predominantly of connective tissue with few scattered inflammatory cells (image adapted from Bahmad and Merchant [119]).

Fig. 8. The concept behind biofabrication: six-step transformation of analytical anatomy (top) into synthetic anatomy (bottom) (images adapted from Mironov et al. [154] © IOP Publishing).

Tables

Table 1. Overview of the use of bioactive glasses for applications in contact with soft tissues, including implantable devices for organ restoration and therapeutic treatments (without the use of drugs or pharmaceutical agents).

Application	Material/implant	Clinical use	Remarks	References
Wound healing	Polymeric sutures coated with Ag-doped bioactive glass, ointments containing bioactive glass powders, fibrous glass or polymer glass composite constructs, composite films with Ag-doped glass.	Yes	Experimented in humans, dogs, rats; some commercial products are available. The ions released for bioactive glasses can elicit an antibacterial, antifungal and/or angiogenic effect, which are very beneficial for wound healing.	[20-52]
Peripheral nerve repair	Phosphate glass tube, phosphate glass fibre wrap, glass micro-/nano-sized fibres (also in hollow form) bundle, micro-/nano-sized phosphate glass	Clinical trials are ongoing	Experimented in sheep and rats; clinical trials ongoing. Glass tubes and wraps can be an effective alternative to microsurgical repair of divided nerve; aligned glass fibres inside a NGC	[56-66,68-73]

	powder/polymer composite		create anisotropic structures to direct cell growth and to potentially release biologically active molecules, thereby stimulating nerve regeneration. Glass powder can improve the mechanical properties in a composite device and release ions that can enhance the nerve healing process.	
Spinal cord repair	Phosphate glass fibres/collagen composite	Not yet (few animal studies are available)	Experimented in rats.	[67,68]
Muscle tissue engineering	Phosphate fibres alone or embedded in polymer-matrix composite constructs.	Not yet (*in vitro* studies with cells are available)	3-D aligned fibre scaffolds were able to support unidirectional muscle cell alignment and caused an up-regulation of genes encoding for myogenic regulatory factors.	[75-78]
Ligament repair	Phosphate fibres	Not yet (*in vitro* studies with cells are available)	The fibres solubility, that can be tuned acting on the F_2O_3 content in the glass composition, strongly affects the	[79]

			proliferation and growth of human osteoblasts and fibroblasts. The proposed application was the *in vitro* simulation of the bone-ligament interface using co-cultures strategies.	
Artificial cornea	Porous glasses or coatings for the keratoprosthetic skirt; glass/PMMA composites	Not yet (few animal studies are available)	Generally good results; promising experiments in rabbits.	[80,81,85-94]
Orbital implants	Porous glass-ceramics, glass fillers of old peg tracts in porous HA implants, glass coatings on pre-existing porous implants (PE or HA)	Yes	Encouraging results (in general). Restoration of damaged peg tracts in old HA implants was successful in humans. Apparently, no significant improvement in implant fibrovascularization was observed in bioactive/glass/PE composite implants with respect to porous PE (studies in both rabbits and humans). Cu-containing MBG coatings on porous HA	[82-84,95-99,101-104]

			implants exhibited antibacterial effect against *Staphylococcus aureus* and *Escherichia coli*.	
Middle ear surgery	45S5 Bioglass® or Ceravital® glass-ceramic structures to replace middle ear ossicles	Yes	Anchoring to both remaining stapes footplate bone and tympanic membrane (collagenous tissue). Commercial products: Bioglass® Ossicular Reconstruction Prosthesis (MEP®) and Ceravital® ossicular chain (prosthetic ossicles made of Ceravital® are currently the unique clinical application of this type of glass-ceramic material). Some issues on the long-term integrity of these implants are under investigation.	[111-118,120-127]
Cochlear implants	45S5 Bioglass® sleeve anchoring the implant through the patient's temporal bone	Yes	Simultaneous bonding to bone and soft tissues. Commercial product: Bioglass®	[128,129]

			Extracohclear percutaneous implant (EPI) (currently almost totally abandoned)	
Treatment of liver cancer	Injectable glass microspheres in the Y_2O_3-Al_2O_3-SiO_2 system	Yes	Commercial product: TheraSphere®	[131-136]
Treatment of uterine fibroids	Injectable glass microspheres in the SiO_2-CaO-ZnO-La_2O_3-TiO_2-MgO-SrO-Na_2O system	Not yet (only *in vitro* tests with cells are currently available)	Cytocompatibility tests with rat fibroblasts.	[139-141]

Table 2. Bioactivity of glasses used for applications in contact with soft tissues.

Application	Effect	Mechanism of action
Wound healing, sutures	Antimicrobial and antifungal action (e.g. inhibition of biofilm formation, inhibition of pathogen replication); angiogenic effect	Release of antibacterial ions (e.g. Ag^{+}. Cu^{2+}, Ga^{3+}) from the glass that interfere with bacterial RNA and DNA replication and/or cause damages to the bacterial cell membrane
Skin regeneration	Angiogenic action	Release of ion dissolution products (e.g. Cu^{2+}) promoting blood microvessels formation
Nerve regeneration	Directional growth of axons on glass fibres; glass fibres resorb as new tissue forms Enhanced nerve healing due to the release of specific ions	Shape effect of the glass fibrous construct (glass fibres orientation directs the cell growth). Release of ions can influence the regulation of the nerve growth cone motility (e.g. Ca^{2+}) and neurotransmission (e.g. Zn^{2+})
Muscle tissue regeneration	Directional growth of muscle cells; glass fibres resorb as new tissue forms	Shape effect of the glass fibrous construct (glass fibre orientation directs the cell growth)
Artificial cornea	Colonization of the keratoprosthetic skirt by keratocytes, inhibition of	Shape effect of the interconnected porosity, the presence of which is a factor that would promote *per se* cell colonization; the mechanisms

	corneal epithelium in-growth in the anterior chamber of the eye	associated to the effects of ions released by the glass on corneal cells have not yet investigated.
Orbital implants	Enhanced fibrovascularization	Shape effect of the interconnected porosity, the presence of which is a factor that would promote *per se* fibrovascular in-growth and blood vessel access (as observed in the case of HA, PE and alumina porous spheres); ions released from the glasses could accelerate the fibrovascularization rate
Middle ear surgery	Bonding to the stapes footplate bone as well as to the tympanic membrane (which is essential for long-term survivability of artificial middle ear ossicles)	Bioactive glasses can bond to both calcified and collagenous tissues
Cochlear implants	Bonding to bone and soft tissues	Bioactive glasses can bond to both calcified and collagenous tissues
Liver cancer	Internal radiotherapy	Injection of radioactive glass microspheres
Uterine fibroid	Embolization of the uterine fibroid	Vessel occlusion and infarction of the leiomyomas

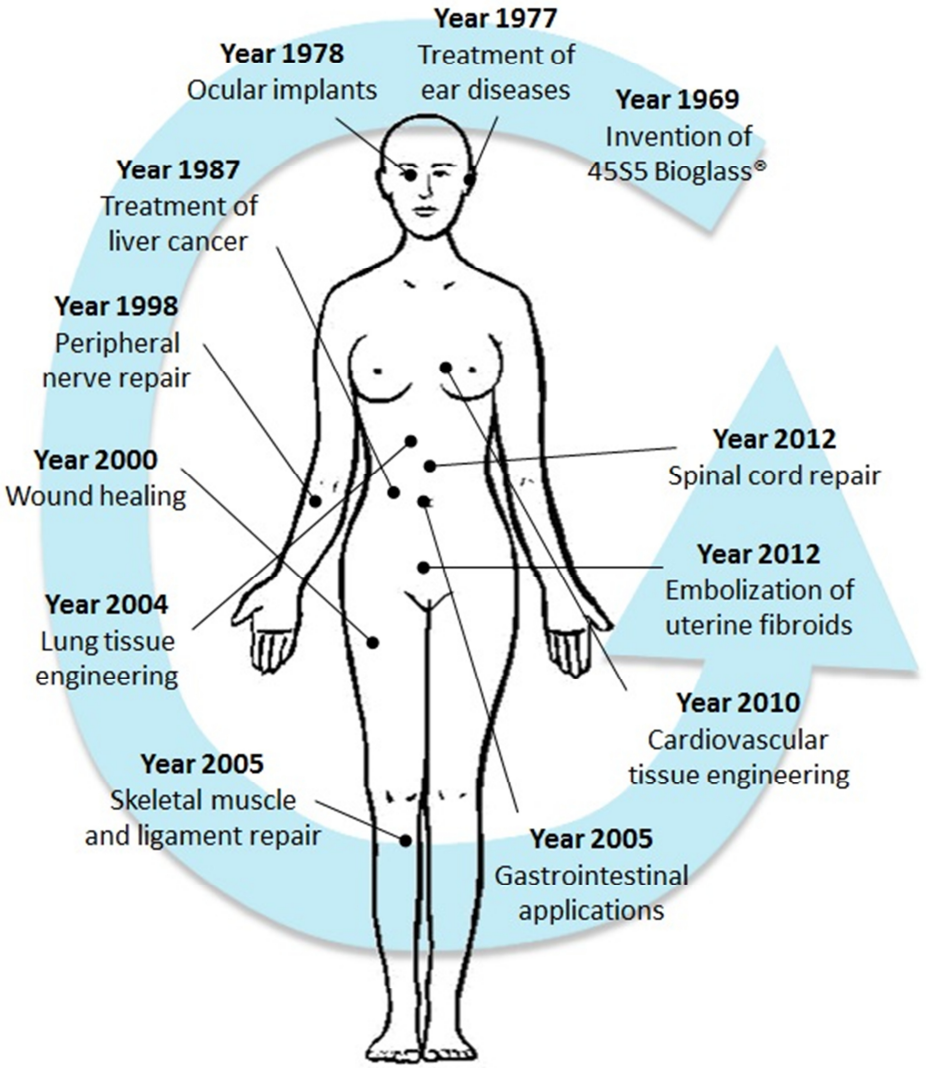
Year 1977
Treatment of ear diseases
Year 1978
Ocular implants
Year 1969
Invention of 45S5 Bioglass®
Year 1987
Treatment of liver cancer
Year 1998
Peripheral nerve repair
Year 2000
Wound healing
Year 2012
Spinal cord repair
Year 2012
Embolization of uterine fibroids
Year 2004
Lung tissue engineering
Year 2010
Cardiovascular tissue engineering
Year 2005
Skeletal muscle and ligament repair
Year 2005
Gastrointestinal applications

Fig. 1

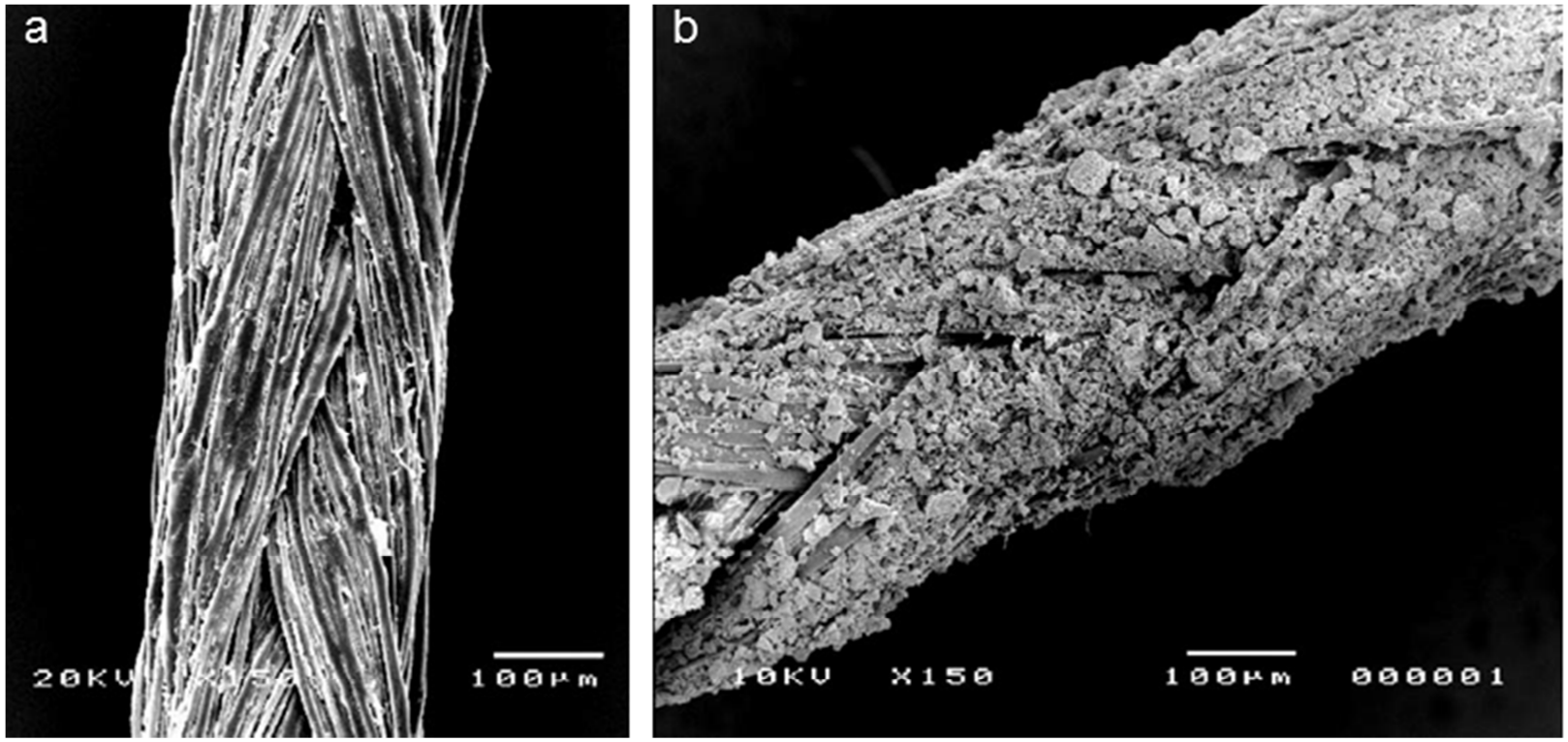

Fig. 2

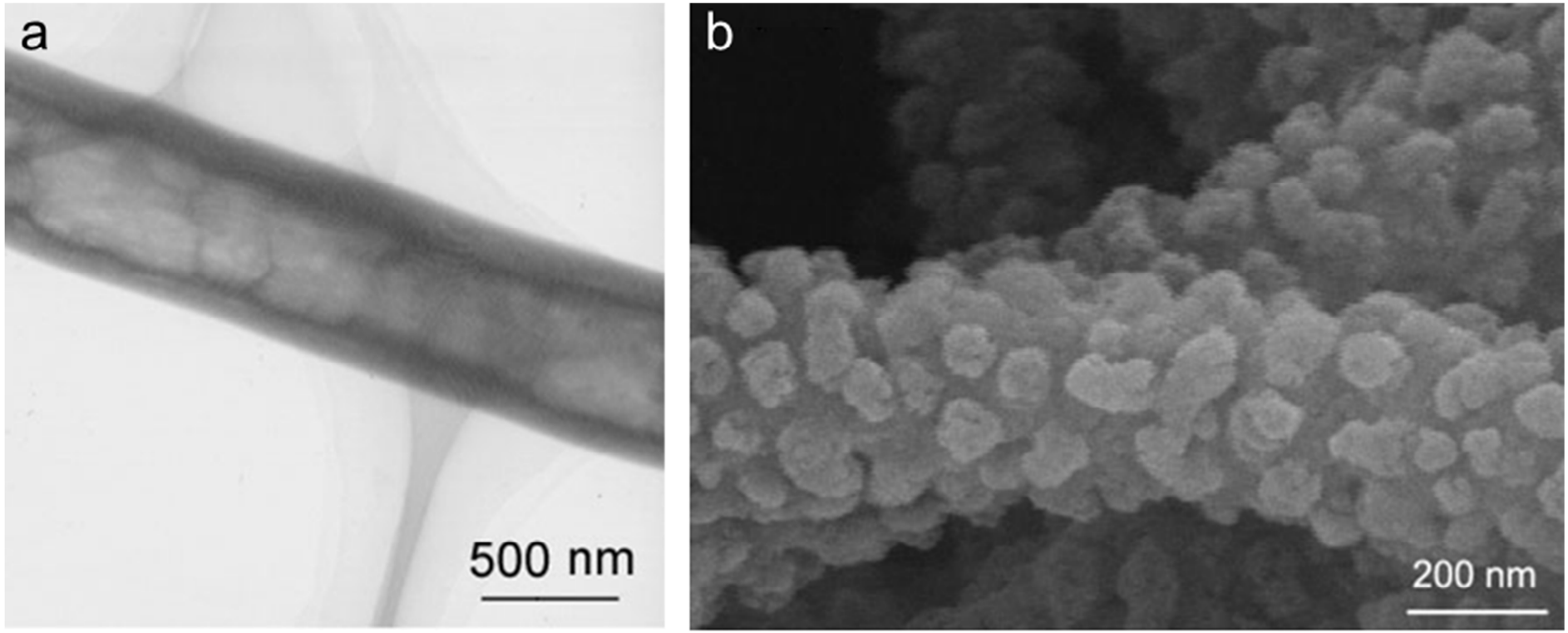

Fig. 3

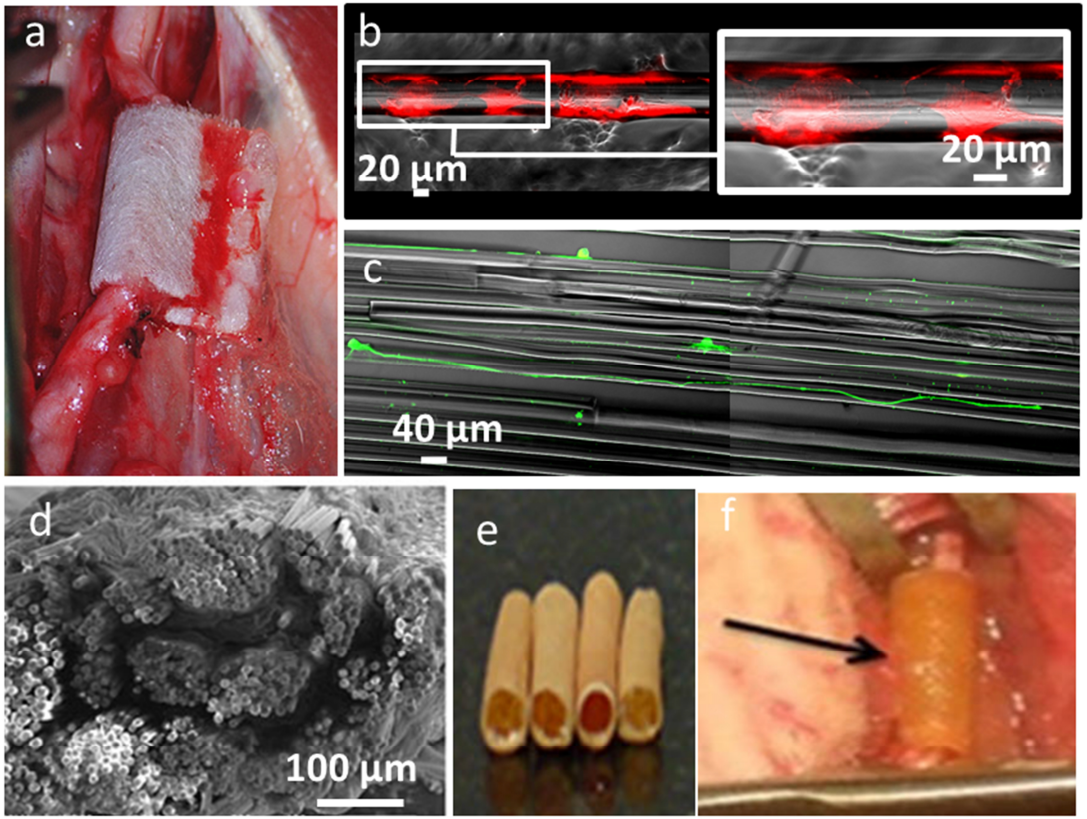

Fig. 4

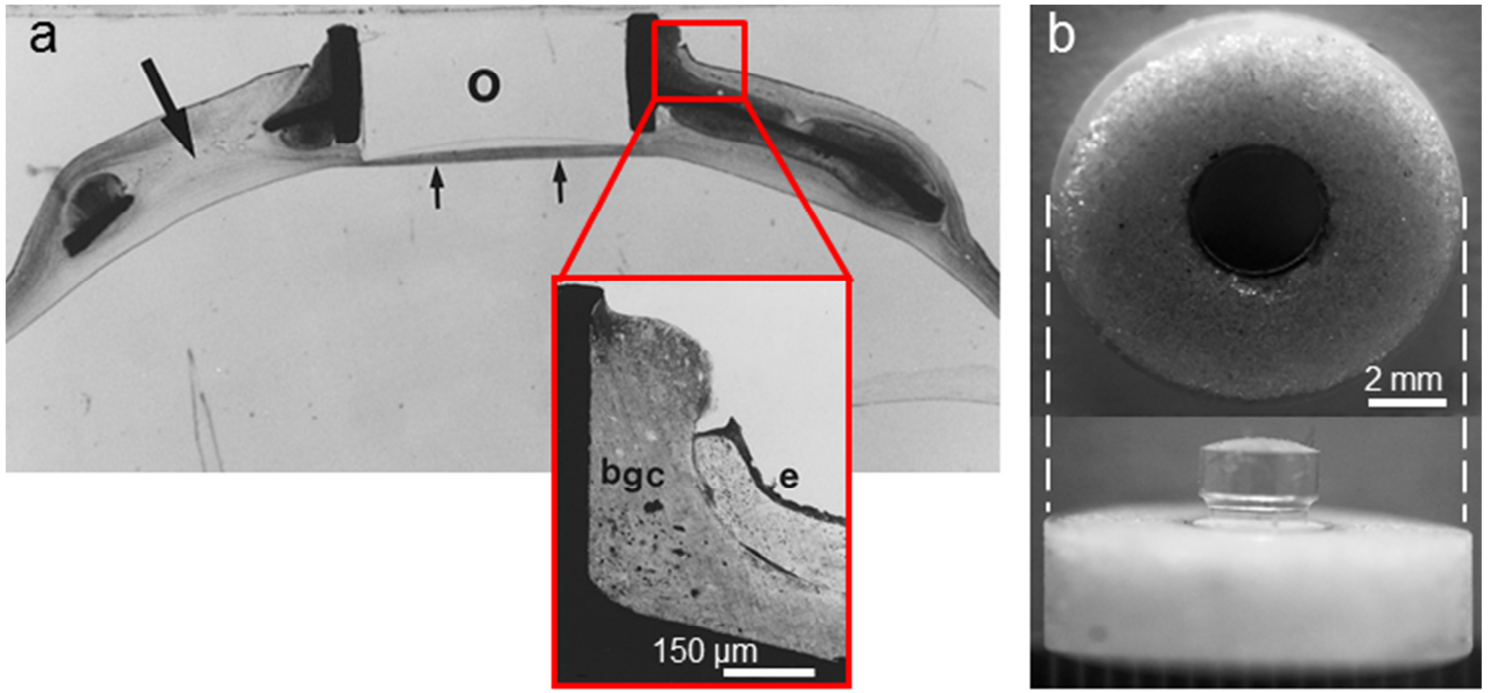

Fig. 5

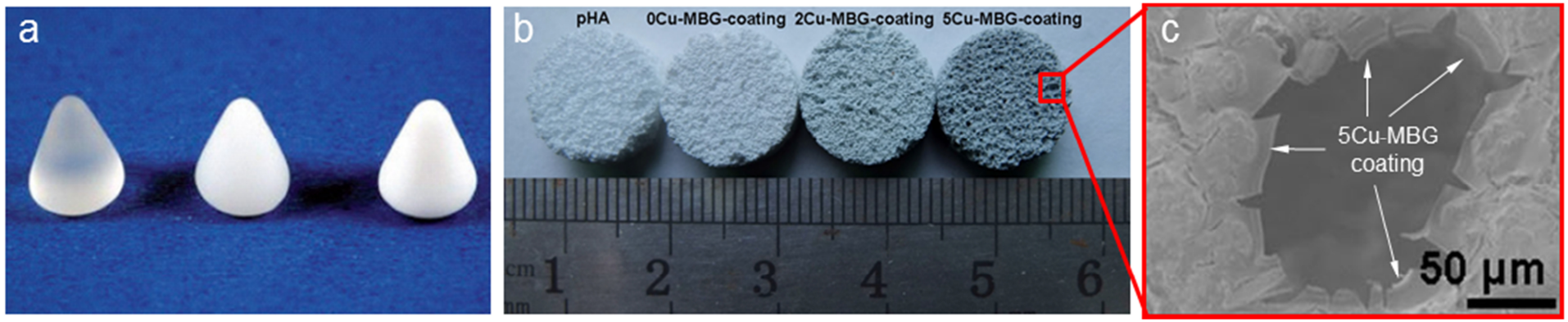
a
b
pHA
0Cu-MBG-coating
2Cu-MBG-coating
5Cu-MBG-coating
cm
1
2
3
4
5
6
c
5Cu-MBG
coating
50 μm

Fig. 6

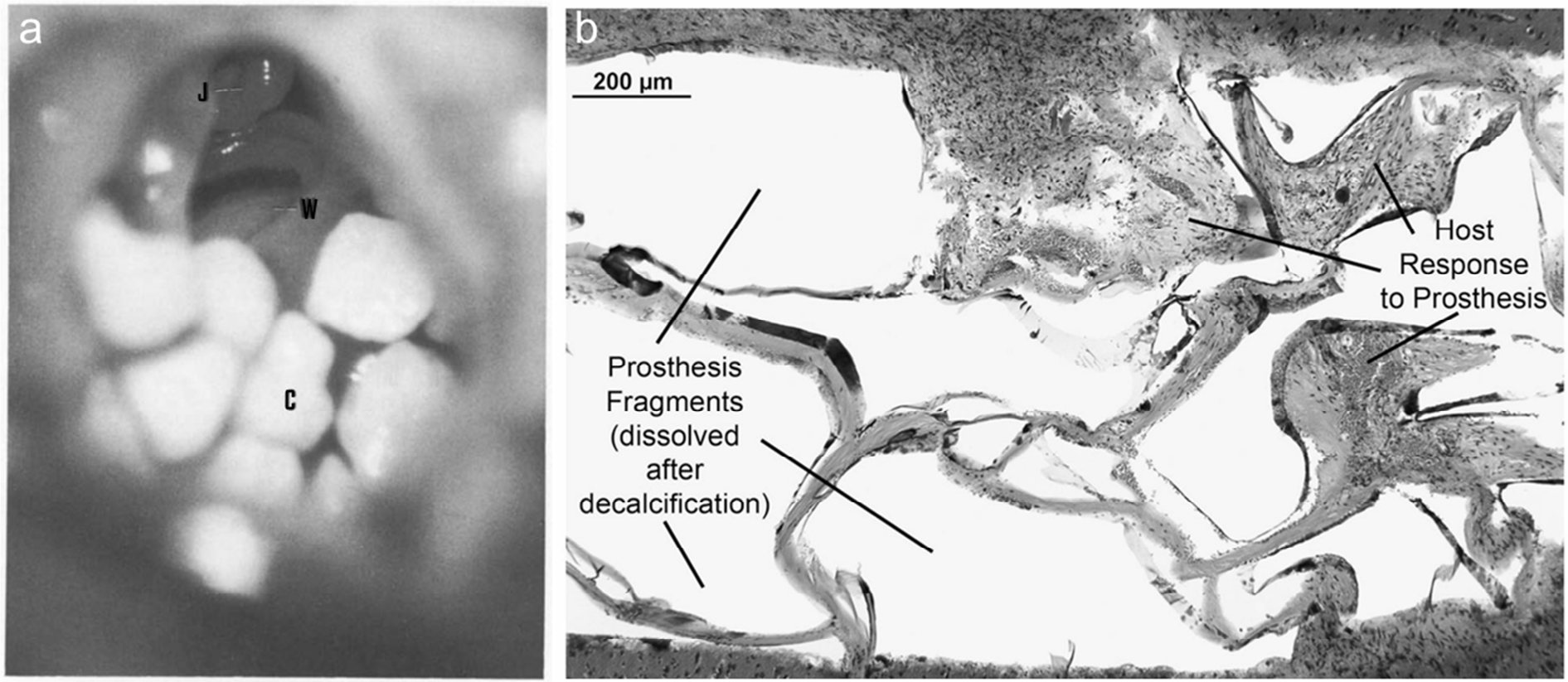
a
J
W
C
b
200 µm
Host
Response
to Prosthesis
Prosthesis
Fragments
(dissolved
after
decalcification)

Fig. 7

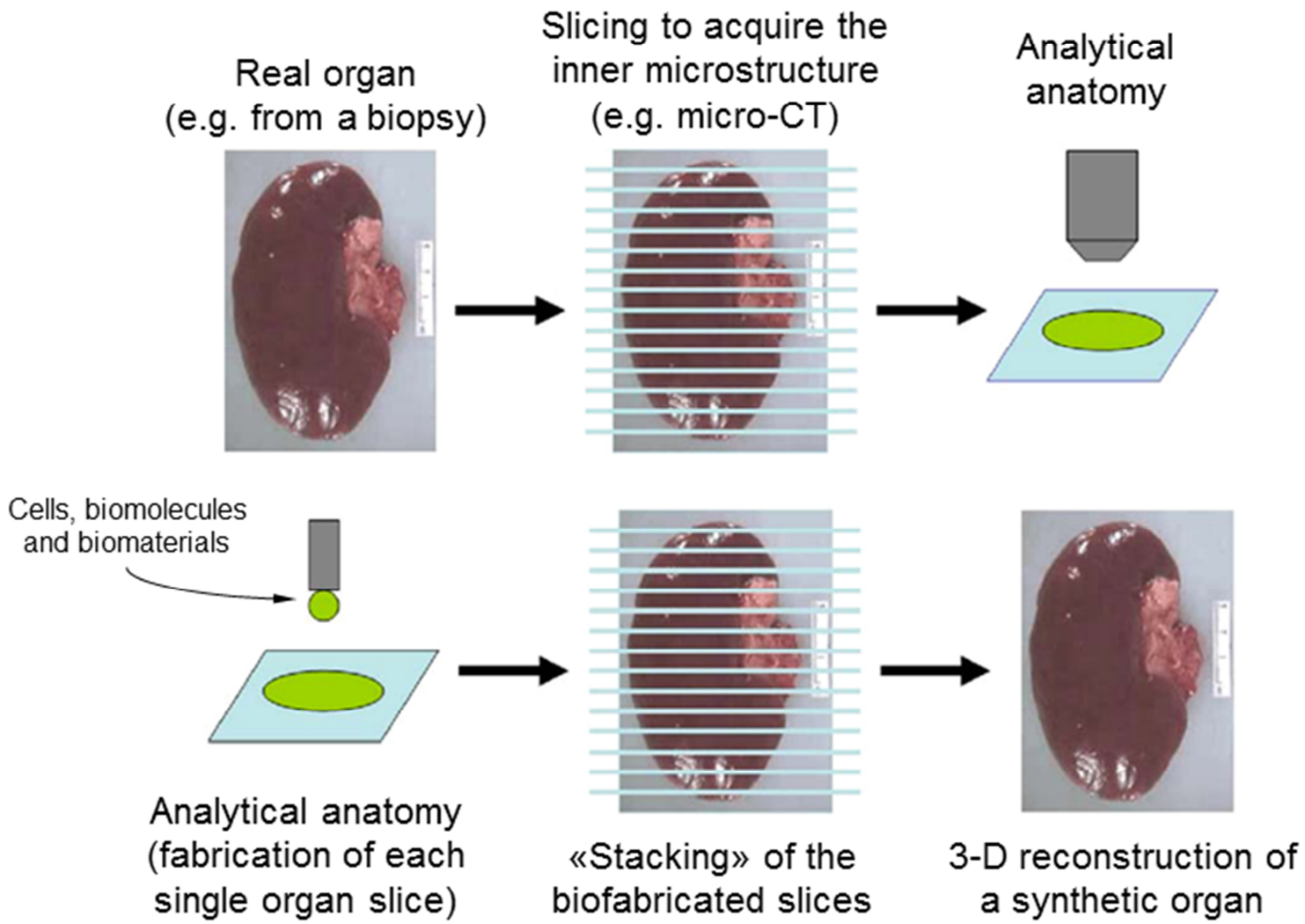
Real organ
(e.g. from a biopsy)
Slicing to acquire the
inner microstructure
(e.g. micro-CT)
Analytical
anatomy
Cells, biomolecules
and biomaterials
Analytical anatomy
(fabrication of each
single organ slice)
«Stacking» of the
biofabricated slices
3-D reconstruction of
a synthetic organ

Fig. 8