Chapter 4

Ceramic Biomaterials for Tissue Engineering

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LIST OF ABBREVIATIONS

ATZ Alumina-toughened zirconia
BMPs Bone morphogenetic proteins
CaPs Calcium phosphates
CPCs Calcium phosphates cements
ECM Extracellular matrix
HA Hydroxyaptite
hBMSCs Human bone marrow stromal cells
MSCs Mesenchymal stem cells
RBMSCs Rat bone marrow stromal cells
β-TCP β-tricalcium phosphate
TE Tissue engineering
TZP Zirconia polycrystals
ZTA Zirconia-toughened alumina

Abstract

Bioceramics, natural and synthetic, are designed to induce a strong bonding to bone and appeared as an alternative to metallic implants. Bioceramic materials currently used for the repair and reconstruction of hard and soft tissues can be categorized according its composition, structure, and properties. These biomaterials are grouped bioinert ceramics as alumina and zirconia, bioactive glasses and glass ceramics and bioresorbable calcium phosphates-based materials. The bioceramics concepts, namely physico-chemical, mechanical and biological properties, and respective applications in diverse fields of tissue engineering are discussed in depth herein. An up-to-date of bioceramics clinical trials is also considered. Based on the stringent requirements for

clinical application, prospects for the development of advanced functional bioceramics for tissue engineering are highlighted for the future.

KeyWords
Bioceramics; Natural-origin bioceramics; Alumina and zirconia; Bioactive glasses and glass-ceramics; Calcium phosphates; Calcium phosphate cements; Bioactivity; Biocompatibility; Bone regeneration; Tissue engineering.

4.1 INTRODUCTION

Bioceramics, natural or synthetic origin, are a class of inorganic and non-metallic ceramics used for repair and regeneration of diseased and damaged parts of the musculoskeletal system and periodontal anomalies. These ceramic materials have been developed for orthopedic load-bearing coatings (hip acetabular cups), bone grafts and cements, and dental implants (1). Bioceramics are typically characterized by their excellent biocompatibility, osteoconductivity, corrosion resistance, and a hard brittle surface. Weaknesses of bioceramics include poor fracture toughness, brittleness, very low elasticity and extremely high stiffness (2). Consequently, their clinical applications for tissue engineering (TE) has been limited. In general, bioceramics are classified as:

- Bioinert: has no interaction with its surrounding tissue after implantation. They have a reasonable fracture toughness, and resistance to corrosion and wear. These ceramics are typically used as structural-support implants, such as bone devices and femoral head. Examples of bioinert ceramics are alumina and zirconia.
- Bioactive: bond directly with living tissues after implantation, with the pattern of bonding osteogenesis. These ceramics are brittle and has been applied for the filling of small bone defects and periodontal irregularities. Examples are bioglasses and glass-ceramics.
- Bioresorbable: gradually absorbed in vivo and is replaced by bone over time. Examples are calcium phosphates (CaPs), calcium phosphate cements (CPCs), and calcium carbonates or calcium silicates.

Current efforts center considerable attention in TE involving bioceramics for developing 3D-based scaffolds able to mimic the structural, mechanical and biological properties of natural tissues (3, 4). Moreover, to stimulate cells differentiation and extracellular matrix (ECM) production during the regeneration process, are also important issues to be considered envisioning the formation of new tissues. Bioceramics are stronger under compression and weak under tension and these facts need to be contemplated when fabricating scaffolds for particular biomedical application. These structures hold porous and fibrous scaffolds, and hydrogels, with defined architecture, controlled degradation rate, and optimized porosity and pore interconnectivity. Scaffold fabrication techniques include sponge replica method, solvent casting and particulate-leaching, freeze drying, gas foaming and phase separation, rapid prototyping and electrospinning (4-11). The latter two

ones are extremely attractive in their ability to mimic new tissue structures and with the possibility of incorporating pharmaceutical agents, even though are expensive and suffer from the materials choice and costs. Additionally, additive manufacturing such as bioprinting and bioinks have presenting a high potential in combination with the design and imaging techniques bringing innovations at the micro- and nano-scale to regenerative medicine (12).

An array of natural and synthetic bioceramics has been proposed to be used in the processing TE scaffolding with specific composition, microstructure and long-term reproducibility. Bioceramics from natural origin, such as corals, nacres, sponges, and animal (fish and chicken) bones, also provide an abundant source of calcium compounds (e.g., calcium carbonate and calcium phosphate) for skeletal TE applications (13). Coral-derived materials has been widely used as raw materials to preparing CaPs biomaterials for bone tissue regeneration, due to their unique microstructural composition and mechanical properties. Our group has been proposing a variety of red algae (e.g., *Coralline officinallis*) to produce porous ceramics aiming bone repair and regeneration (14, 15). This process involves the conversion of calcium carbonate skeletons of *C. officinallis* particulates into CaPs with hydroxyapatite (HA) nanocrystallites, while maintaining the native microstructure of the red algae, using a thermal and chemical treatment (14).

Concerning synthetic bioceramics, alumina and zirconia, bioactive porous glasses and glass-ceramics, and CaPs-based materials in the form of sintered ceramics, coatings and cement pastes, are the ones mostly used in TE applications (16, 17). These bioceramics can be prepared by several methods (e.g. wet precipitation, hydrolysis, sol-gel synthesis, hydrothermal synthesis, mechanochemical synthesis, microwave processing, or spray drying methods) yielding materials with different properties, such as crystal size and morphology. Among them, wet precipitation method has the advantage on the homogeneity of the final product, and the easiness of controlling parameters (e.g. temperature, pH, and the presence of additives) during synthesis (18).

Many studies are dedicated on bioceramic materials incorporating ionic elements (e.g. strontium, zinc, magnesium, manganese, silicon) that would be released during bone graft resorption, and hence can influence bone health and enhance biocompatibility, while strengthening the mechanical properties of the implants (19-23). Besides, minerals and traces of metal elements may provide physicochemical modifications in the produced materials, which can accelerate bone formation and resorption *in vivo* (24, 25).

This chapter aims at presenting a concise and reachable overview of bioceramics for applications in the contexts of musculoskeletal and periodontal tissue regeneration. A range of materials are considered, from bioinert to bioactive and bioresorbable bioceramics. Particularly, emphasis is set on synthetic bioceramics physicochemical, mechanical and biological properties. Clinical trials involving bioceramics, challenges and future prospects of research in this field are also underlined.

4.2 BIOCERAMIC MATERIALS CONCEPTS

4.2.1 Alumina and Zirconia

Alumina (Al_2O_3) and Zirconia (ZrO_2) are well known for their general chemical inertness, high strength, hardness, cracking, and corrosion resistance, thus being recognized as bioinert ceramics successfully used in orthopedics, specifically for total hip/knee arthroplasty**,** and in dental repair/replacement (Figure 1) (26, 27).

Alumina-based bioceramics were the first commercially available for dental implantation and acetabular cup replacement in total hip prostheses (28). Alumina favorably combine high hardness and high abrasion resistance, associated with its surface energy and smoothness. Hence, this bioceramic has been used as synthetic bone grafts or as porous prosthetic device, by using a biomimetic coating on alumina in order to provide a stable bond with the host tissue. Further clinical applications of alumina prostheses include bone screws, alveolar ridge (jaw bone) and maxillofacial reconstruction, ossicular (middle ear) bone substitutes, corneal replacements, segmental bone replacements, and blade and screw and post-type dental implants (29). However, the alumina ceramics have low fracture toughness, which can be significantly improved by adding zirconia (known as zirconia-toughened alumina (ZTA) or alumina-toughened zirconia (ATZ)) resulting in a composite material with enhanced toughness and tribological properties (30, 31). ZTA comprises alumina (70–95%) matrix phase and zirconia polycrystals (TZP) (5-30%) as the secondary phase, thus combining the advantageous properties of monolithic alumina and zirconia. In addition, the wear properties and low susceptibility to stress-assisted degradation of alumina ceramics is also preserved in ZTA ceramics, reducing the risk of impingement and dislocation, and improving stability (31).

In consequence of its polymorphic crystalline structure – monoclinic, tetragonal, and cubic - zirconia-based bioceramics, namely tetragonal TZP, have been widely popular in bone TE, due to their excellent fracture toughness, high strength, elastic modulus, and wear resistance (32). For example, partially stabilized zirconia (with yttria, CaO, and MgO) materials are known to have flexural strength above 1,000 MPa and fracture toughness higher than 8 $MPam^{1/2}$ (33, 34). Besides its mechanical properties, zirconia promotes cell proliferation and differentiation in osteogenic pathways, as well as osseointegration, and has radiopaqueness that helps the monitoring in radiographs (35). Zirconia has often been used in dentistry since it has the advantage of being coloured to match the shade of any existing teeth.

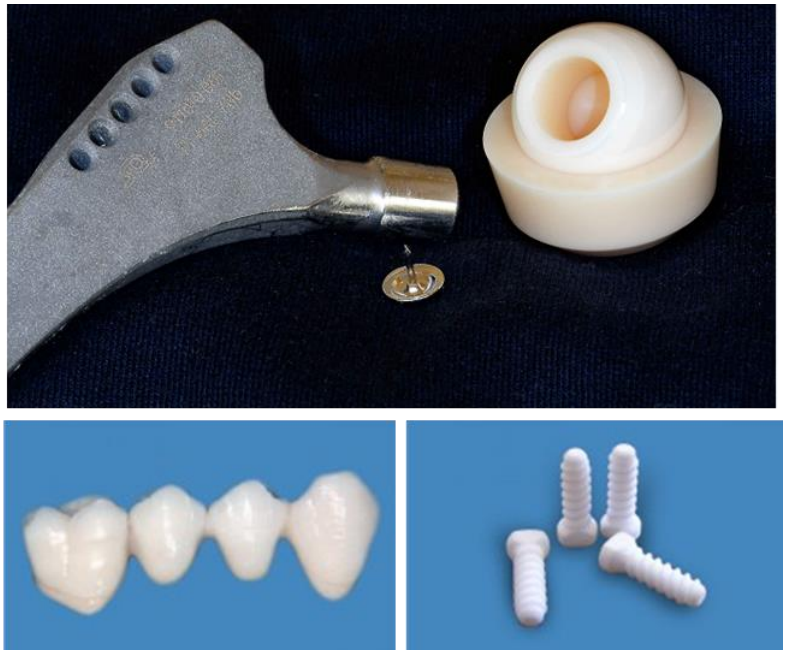

FIGURE 1. Examples of alumina/zirconia bioceramics for hip joint prosthesis and dentistry (36, 37).

4.2.2 Bioactive glasses and glass-ceramics

Bioactive glasses and glass–ceramics have been developed for TE applications, in both orthopedic and dental field, in dense and porous form (Figure 2) (38-40). Glass-ceramics are crystallized glasses resultant from thermal treatment of its parent glasses, with superior strength and toughness, elastic modulus, and wear resistance.

Bioactive glasses have unique properties with ability to bond to both hard and soft connective tissues more rapidly than other bioceramics, converting into an amorphous calcium phosphate or hydroxyapatite material after implantation. Moreover, it is also reported that the ions Si, Ca, P and Na, released during dissolution of certain bioactive glasses compositions appear to activate expression of osteogenic genes, and to stimulate neovascularisation and angiogenesis, enzymatic activity, and differentiation of mesenchymal stem cells (MSCs) (41-43).

The pioneering work in the field of bioactive glasses, for biomedical applications, data from the beginning of the 1970s with the development of 45S5 Bioglass® by Larry Hench (29). Bioglass®, is a silica-based bioactive glass in the Na_2O-CaO-SiO_2-P_2O_5 system with a composition close to a ternary eutectic in the Na_2O–CaO–SiO_2 diagram. This type of glass has also the particularity of stimulates bone growth away from the bone–implant interface, which mechanism can be attributed to a hydroxycarbonate apatite layer on the surface of the glass (40, 44, 45).

Besides silicate glasses, phosphate-based and borate-based glasses are other types of bioactive glasses developed for biomedical use. Phosphate bioactive glasses, in the Na_2O-CaO-P_2O_5 system, have faster dissolution in aqueous fluids, than that for silica glasses, useful in the healing of chronic wounds and as carriers in drug delivery such as antibacterial ions and complex organic molecules

for chemotherapy applications (46, 47). By its turn, borate-based glasses, in the B_2O_3-Na_2O-CaO-P_2O_5 system, have fast degradation rates and are able to completely convert into apatite when immersed in an aqueous phosphate solution following a similar process of Bioglass®, but without the formation of a silica-rich layer (48, 49). Borate glasses have been also used as drug release systems in the treatment of bone infection (50). A concern associated with these type of glasses is the toxicity of boron released into the solution as borate ions, which can be reduced in *in vitro* dynamic culture conditions (51).
The common methods of synthesis of bioactive glasses include conventional melt-quenching, sol-gel process, flame synthesis and microwave irradiation (52, 53).

FIGURE 2. Bioactive glass and glass-ceramics for biomedical applications and porous robocast bioglass produced at Missouri University of Science and Technology for bone repair and regeneration (*) (54, 55).

4.2.3 Calcium phosphates

Calcium phosphates (CaPs) are the chemical compounds of special interest for TE applications due to their close resemblance with the inorganic part of major normal and pathological calcified tissues of mammals (56-58). These types of bioceramics hold an outstanding biological performance, such as biocompatibility, osteoconductivity and bioresorbability, thus integrating into living tissue by the same processes active in bone remodeling. Besides, CaPs are easy to produce with a low cost, and can be relatively easily certified as medical grade. Despite that, CaPs are limited to load-bearing applications due to their poor mechanical properties, namely, strength and fatigue resistance, being primarily used as fillers and coatings in the biomedical field (56, 59). However, CaPs bioceramics are also available in particles, dense or porous blocks, injectable compositions, implant coatings, and composites with polymers (Figure 3). Custom-designed forms as wedges for tibial opening osteotomy, cones for spine and knee, and inserts for vertebral cage fusion, are also available. CaPs are used in alveolar ridge augmentation, tooth replacement, maxillofacial reconstruction, orbital implants, increment of the hearing ossicles, spine fusion and repair of bone defects (60).

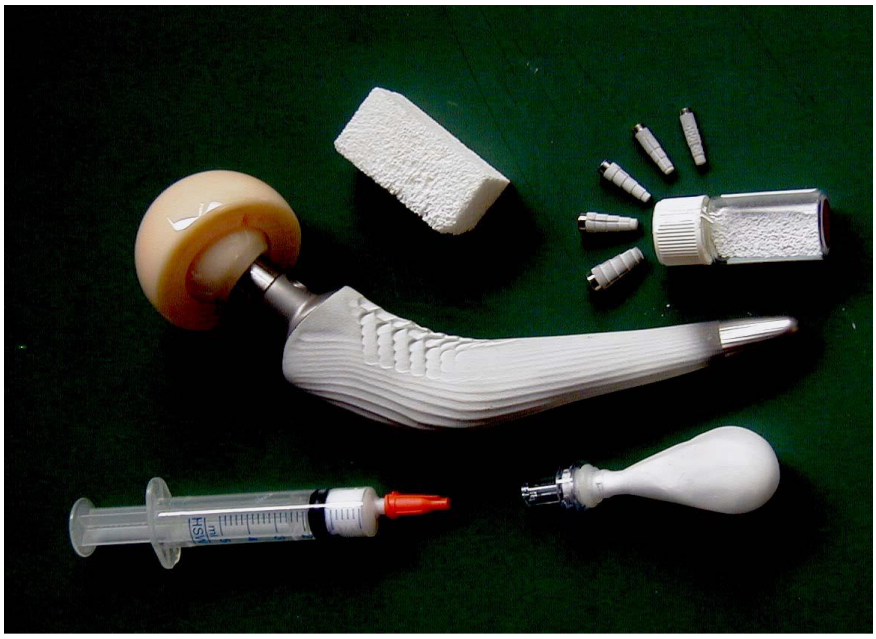

FIGURE 3. Commercial calcium phosphate-based bone graft materials, such as porous blocks, powders and granules, and HA coating on femoral metal stem. *Reprinted from Ref. (57), with permission.*

The most known CaPs, listed in Table 1.1, comprise Ca/P molar ratios in the range of 0.5 - 2, depending on their acidity and solubility. CaP is more acidic and water-soluble for lower Ca/P molar ratios (61). The majority of CaPs are sparingly soluble in water, but, all of them are easily soluble in acids but insoluble in alkaline solutions. According to solubility, CaPs can be ranked in order of increasing the *in situ* degradation rate as: MCPM > TTCP ≈ α-TCP > DCPD > OCP > β-TCP > HA.

Table 1.1: Main calcium phosphates used for biomedical applications (3, 57).

Calcium phosphate	**Formula**	**Ca/P molar ratio**	**pH stability range in aqueous solutions at 25°C**	**Properties**
Monocalcium phosphate monohydrate (MCPM)	$Ca(H_2PO_4)_2.H_2O$	0.5	0.0 – 2.0	Not biocompatible
Monocalcium phosphate anhydrous (MCPA)	$Ca(H_2PO_4)_2$	0.5	Stable at T >100°C	
Dicalcium phosphate dihydrate (DCPD)	$Ca(HPO_4).2H_2O$	1.0	2.0 – 6.0	Biocompatible, biodegradable and osteoconductive
Dicalcium phosphate anhydrous (DCPA)	$Ca(HPO_4)$	1.0	Stable at T >100°C	
Octacalcium phosphate (OCP)	$Ca_8(HPO_4)_2(PO_4)_4.5H_2O$	1.33	5.5 – 7.0	Metastable precursor of CaPs that transforms into HA
Amorphous calcium phosphate (ACP)	$Ca_xH_y(PO_4)_z.nH_2O$ (n = 3 - 4.5)	1.2 - 2.2	~ 5 – 12 (Always metastable)	Lacks long range order
Calcium-deficient hydroxyapatite (CDHA)	$Ca_9(HPO_4)(PO_4)_5(OH)$	1.5 – 1.67	6.5 – 9.5	Poorly crystalline
β-Tricalcium phosphate (β-TCP)	β-$Ca_3(PO_4)_2$	1.5	Cannot be precipitated from aqueous solutions	Biodegradable
α-Tricalcium phosphate (α-TCP)	α-$Ca_3(PO_4)_2$	1.5	Cannot be precipitated from aqueous solutions	

Hydroxyapatite (HA)	$Ca_{10}(PO_4)_6(OH)_2$	1.67	9.5 – 12	Osteoconductive
Tetracalcium phosphate (TTCP)	$Ca_4(PO_4)_2O$	2.0	Cannot be precipitated from aqueous solutions	Biocompatible but poorly biodegradable

Attention in the biomedical field is generally focused on HA, α- and β-TCP, CDHA and biphasic CaPs, since implants made of calcined HA stay in bone defects for many years upon implantation (56, 62). HA is crystalline and is the most stable and least soluble CaPs in an aqueous solution below pH 4.2 (56). HA can be produced using wet methods, such as precipitation method, hydrothermal synthesis and solid-state reaction of, for example, MCPM, DCPA, DCPD, OCP, above 1200ºC (63-66). β-TCP is a high temperature phase of CaPs, obtained by thermal decomposition at temperatures above 800ºC. β-TCP is biodegradable and has been extensively used as bone substitute, either as granules or blocks, or even in CaPs-based bone cements (57). It has been reported that the biological resorption capability of HA and β-TCP is different though their similarity in terms of chemical composition. HA has a slow resorption rate and may remain integrated into the regenerated bone tissue after implantation, whereas β-TCP is completely reabsorbed (67, 68). Therefore, clinical applications have been performed using the biphasic CaPs, as a result of combining HA and β-TCP, thus improving the bioresorbability and strength of the bone substitutes (62, 66, 69). α-TCP is usually prepared from β-TCP phase at heat treatment above 1125ºC, and quenching it prevents the reverse transformation (70). α-TCP is biocompatible, and more biodegradable and reactive than β-TCP (71). CDHA is obtained by precipitation in an aqueous solution above a pH 7 (56). Their crystals are in general poorly crystalline and of submicron dimensions. The solubility of CDHA increases with a decrease of Ca/P molar ratio, crystallinity and size. CDHA can decompose into β-TCP, into a mixture of HA and β-TCP or into pure HA, when heating above 700ºC (62, 72). As a first approximation, CDHA may be considered as HA with some ions missing (73).

4.2.3.1 Calcium phosphates-based cements

In 1832, Ostermann prepared a CaP biomaterial in the form of a paste that set *in situ* to form a solid material. Nevertheless, Brown and Chow in 1986 (74) were the first to present this new form of CaPs, currently known as calcium phosphate-based cements (CPCs).

CPCs result from the mixture of one or several CaPs and an aqueous solution, which then precipitate into a less soluble CaP and sets by the entanglement of the growing crystals, providing mechanical stiffness to the cement, and then, the paste can be placed into the bone defect (Figure 4). Subsequently it hardens in situ, at body temperature, and then displays limited solubility.

CPCs salient features are excellent biocompatibility and resorbability, bioactivity, non-cytotoxicity, development of osteoconductive pathways and sufficient compressive strength for a number of applications (56, 67, 75, 76). CPCs are mechanically much stronger in compression than in tension or shear, because entangled crystals are not well bonded. Compressive strength values are typically 5–10 times larger than that of tensile strength. The foremost advantages of the CPCs include fast setting, excellent mouldability and manipulation. Hence, these bioceramics are commonly used to fill bone defects and trauma surgeries as mouldable paste-like bone substitute materials. Besides, like any other bioceramics, CPCs provide the opportunity for bone grafting using alloplastic materials, which are unlimited in quantity and provide no risk of infectious diseases.

CPCs can be classified according to their end product into apatite (AP) cements and dicalcium phosphate dehydrate (DCPD or brushite) cements, upon the pH value of a cement paste after setting. AP is formed above pH 4.2, whereas brushite is preferentially formed when pH value of the paste is < 4.2, although it may grow even up to pH 6.5, due to kinetics reasons (77, 78). Brushite cements have raised interest due to their higher solubility and resorbability *in vivo* much faster than AP cements. Although AP cements show higher mechanical strength, they have slow in vivo resorption rates that interfere with the bone regeneration process (79, 80). Moreover, brushite-based cements possess faster setting reactions (19, 81).

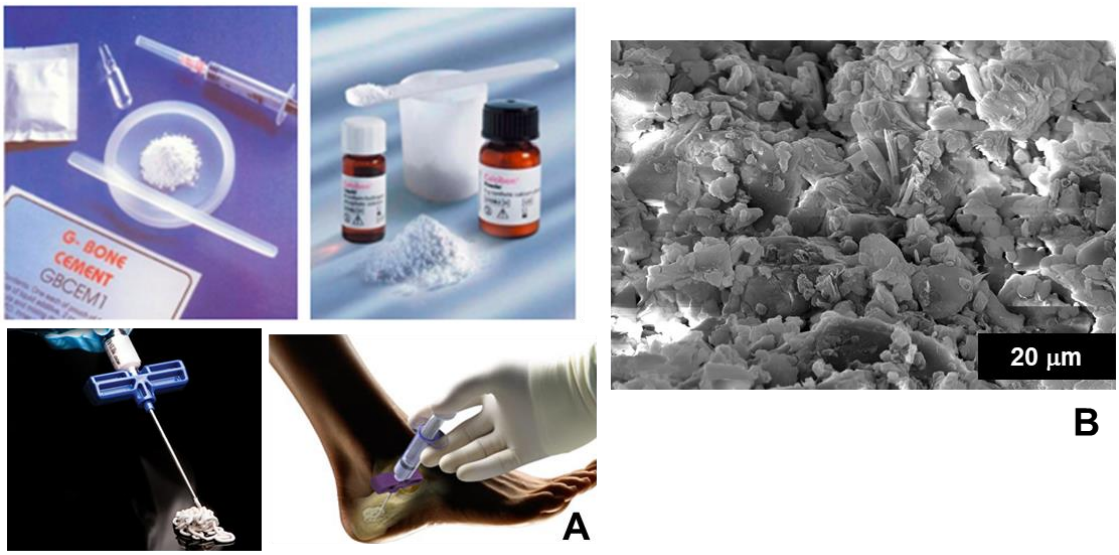

FIGURE 4. A) Self-setting CPCs pastes resultant from CaPs powders and an aqueous solution that then can be injected into the bone defect; and B) Brushite cement microstructure after hardening, showing entangled growing crystals, which provides the mechanical stiffness to the cement (82, 83). *Reprinted from Ref.(83), with permission.*

4.3 BIOCERAMICS APPLICATIONS IN TISSUE ENGINEERING

A diversity of clinical procedures using bioceramics include bone grafting, drug delivery, gene transfection, and for bone cancer treatment (84-86). Alongside is the possibility to combine them with bioactive signaling molecules and/or stem cells that provide important cues and signals promoting cells adhesion, proliferation, differentiation, and metabolic activity for the *in vivo* regeneration process. These bioactive molecules encompass growth factors (with proliferation-inducing effects), mitogens (that stimulate cell division), and morphogens (that control generation of tissue form). Bone morphogenetic proteins (BMPs) growth factors are the most significantly used for bone growth and healing, namely in spinal fusion, long bone defects, and oral and maxillofacial surgery, due to their osteoinduction ability (87, 88). Stem cells (human MSCs, human bone marrow stromal cells (hBMSCs), human endometrial stem cells, adipose-derived stem cells) have promising outcome of functional bone recovery, with good implant integration and host bone formation post-surgery (89). For instance, an interesting recent study reported that CPC-based scaffold combining mesoporous silica with recombinant human BMP-2 (rhBMP-2) might provide a solution to issues of tissue necrosis during the regeneration process by facilitating vascularization and osteogenesis (90). The scaffolds induced the osteogenic differentiation of hBMSCs and demonstrated abundant new vessel formation, as well as rapid rates of osteogenesis *in vivo* owing to the collaborative effects of the biomaterials and growth factor.

Currently there is a range of ceramic products made of alumina/zirconia, bioactive glass and glass-ceramics, and CaPs-based implants as porous and fibrous scaffolds, and hydrogels (60, 91, 92). For instance, Oliveira et al. (93) prepared porous HA scaffolds with highly interconnectivity, using an organic sacrifice template, for bone TE (Figure 5). *In vitro* cell/material interaction tests using rat bone marrow stromal cells (RBMSCs) demonstrated that the cells adhered, proliferated well and remained viable on the scaffolds.

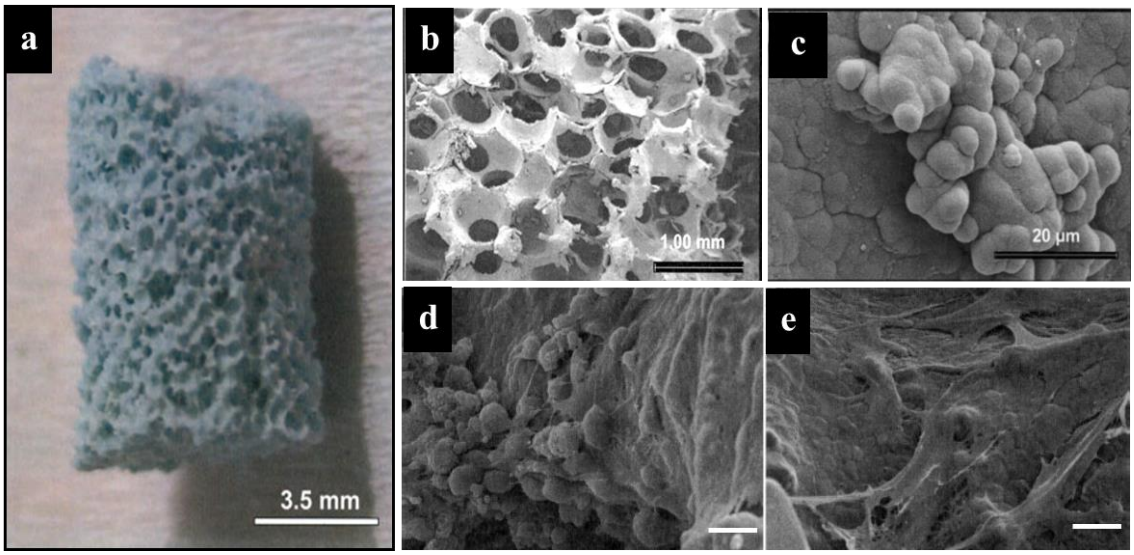

FIGURE 5. HA porous scaffold: (a) macroscopic image, (b) microstructure, (c) microstructure after immersion in SBF for 7 days showing the ‘‘cauliflower like’’ morphology of apatite formed on the scaffold surface, and microstructure showing RBMSCs seeded after culturing for (d) 24 h and (e) 7 days (scale bar: 10 μm). *Reprinted from Ref. (93), with permission.*

Mainly of these bioceramic implants are used in non-load-bearing applications, or compressive load situations, owing to their biomechanical limitations, namely as bone fillers of defects in reconstruction surgery, middle ear repair, vertebral, and iliac crest replacements (57, 58, 94). However, bioceramics-based composite scaffolds have appear as an alternative to circumvent this drawback and to be used to engineer hard tissues. An example is the production of CaPs-based composite scaffolds showing worthy mechanical properties and stability, and self-mineralization capability without cytotoxicity for bone TE (4, 93). Our group has been proposed composite porous scaffolds using CaPs and biodegradable and biopolymeric matrices (i.e., proteins, polysaccharides, and glycosaminoglycans) as a strategy for TE and regeneration (4, 6, 7, 95). For example, silk fibroin/nanosized CaPs composite scaffold provided an optimal microenvironment in terms of porosity and pore interconnectivity, and physicochemical structure, with self-mineralization capability and no cytotoxicity (Figure 6) (4, 96). Further, the incorporation of CaP in the silk fibroin matrix promoted the attachment, viability, and proliferation of the hASCs (96).

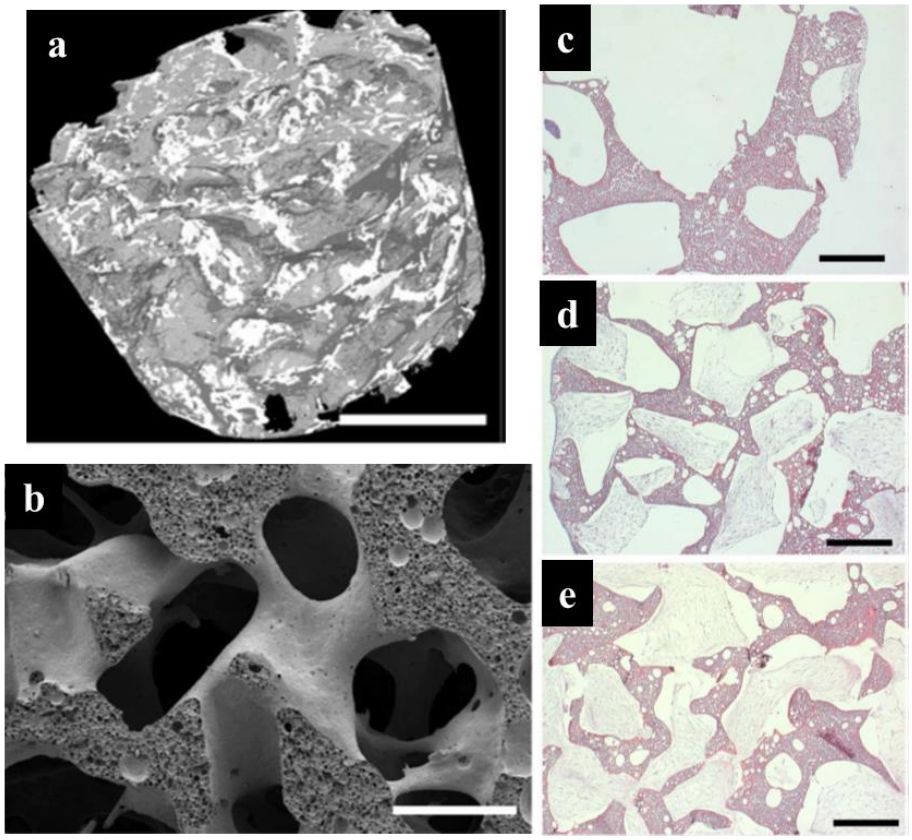

FIGURE 6. Silk fibroin/CaPs composite scaffolds: (a) 3D Micro-CT image showing CaP (white region) and silk fibroin matrix (gray region), (b) microstructure, and (c)-(e) H&E staining of hASCs cultured on the scaffolds for 3, 7, and 14 days, respectively. Scale bar: 500 mm. *Reprinted from Ref.(96), with permission.*

Extensive studies have also reported bioactive glass ceramic-based composites for the regeneration of hard and soft tissues. For instance, it was shown that a porous tri-layered nanocomposite scaffold composed of chitin poly (lactic-co-glycolic acid)/nano bioactive glass ceramic/cementum protein 1 as the cementum layer, chitin- poly (lactic-co-glycolic acid)/fibroblast growth factor 2 as the periodontal layer, and chitin- poly (lactic-co-glycolic acid)/nano bioactive glass ceramic/platelet-rich plasma derived growth factors as the alveolar bone layer, is cytocompatible and favored cementogenic, fibrogenic, and osteogenic differentiation of human dental follicle stem cells (97). The scaffold with growth factors demonstrates complete defect closure and healing with new cancellous-like tissue formation and formation of new cementum, fibrous periodontal tissue, and alveolar bone with well-defined bony trabeculae in comparison to the other three groups, upon implantation into rabbit maxillary periodontal defects. Gantar et al. (98) prepared gellan-gum hydrogels reinforced with bioglass to improve the microstructure and the mechanical properties of the biomaterial for bone TE. The hydrogels exhibited an open and well-interconnected porosity of ~80 % and a pore size of ~100-200 μm, recommended for bone TE scaffolding. Moreover, the ions released from the bioglass conferred the possibility to mineralize in vitro when combined with adipose stem cells.
An overview of bioceramics for varied TE purposes are summarized in Table 1.2.

TABLE 1.2 Summary of bioceramics in different TE applications.

Application	**Bioceramic materials**	**Function**	**References**
Maxillofacial surgery	Bioglass®, HA and β-TCP scaffolds, biphasic CaPs; self-setting CPCs	Repair/replacement of lost teeth; filling of jaws defects; reconstruction of mandible and temporomandibular joint	(94, 99)
Orbital surgery	Alumina, HA, Bioglass®	Improving prosthesis motility resulting in a very natural-appearing eye; orbital floor fractures repair	(100, 101)

Dental surgery	Alumina, zirconia, bioglass, HA	Replace diseased, damaged or loosened teeth	(102)
Periodontal regeneration	Alumina, zirconia, HA and β-TCP nanoparticles, and bioactive glasses	Promote enamel, dentin and periodontium healing; differentiation and proliferation of ameloblasts, odontoblasts, cementoblasts, osteoblasts and fibroblasts	(88, 103)
Lung tissue engineering	Bioglass® composite	Adhesion and proliferation of human lung epithelial type II cells	(104)
Joint arthroplasty	Alumina, zirconia, bioglasses and HA coatings of acetabular cup	Osteoconduction and osteointegration of prosthetic devices; To reduce wear and inflammatory response	(105)
Bone defects and diseases	Bioglass, CaPs, and CPCs	Filling bone defects; Repair and regeneration of damaged bone	(25, 53, 106)
Spinal surgery	Zirconia, bioglass, and CPCs	Immobilize vertebrae to protect spinal cord; high compressive strength	(107, 108)
Wound healing	Silver doped bioactive glass, Bioglass®	Bioactive, antimicrobial and bactericidal properties to the sutures	(109)
Cosmetics	Bioglass (Vitryxx®, Schott AG)	Antiaging benefits, such as reducing redness and wrinkles	(110)

4.4 CLINICAL TRIALS

Human clinical research studies conducted around the world are designed to answer specific questions about biomedical or behavioral interventions, including new treatments and known interventions that warrant further study and comparison. Clinical trials give data on products safety and efficacy and are only conducted after approval of the health ethics committee. Table 1.3 provides the completed and ongoing (with no reported results so far) clinical research trials for TE applications using different types of bioceramics.

The process of the products commercialization for implantation involves multiple stages of R&D replications before reaching the final stages of approval from the governing bodies. R&D stages ensure efficacy and safety of the devices, involving the fabrication of medical grade scaffolds followed by animal testing under regulatory approved conditions. The FDA provides regulatory

guidance and approval for biomaterials and devices and classified them according the associated risk. Fracture fixation devices are classified as Class 2 - medium risk, while devices for organs replacement, such as heart valves, are Class 3 (111).

Up to now, for bone regeneration there are no tissue engineered approaches fully approved for clinical application. Instead, just engineered materials/scaffolds already regulatory approved are arriving in the clinic as bone grafts (without the combination of cells), such as Infuse® Bone Graft (Medtronic Sofamor Danek) used for fusion of spinal cage, Osigraft (Stryker Biotech) for long bone non-unions applications, and Grafton® Orthoblend (OsteoTech) as a bone void filler for small and large defects, have been successfully reported. Despite their efficacy in bone regeneration, clinical translation of scaffold-based bone therapies is limited to small defects due to insufficient mechanical integrity.

A remarkable and largest commercial is the use of bioactive glass in toothpastes. A bioglass 45S5 particulate, named NovaMin® (NovaMin Technology, FL, owned by GlaxoSmithKline, UK since 2010) and fluoride-releasing bioactive glass, denominated BioMinF® (BioMin Technologies Ltd, London, UK) were designed to promote a partial remineralization of a demineralized enamel, as well as, a whitening effect and reduce tooth sensitivity (110, 112).

TABLE 1.3 List of completed and ongoing clinical trials using bioceramics for TE applications. Information obtained from https://clinicaltrials.gov/.

NCT number	Date and status	Study	Patients age	Follow-up	Procedure
NCT00200603	2005	Autograft Versus Calcium Phosphate Macroporous Bioceramics as Bone Substitute for Tibial Valgus Osteotomy	Adult and senior	n.d.	Tibial valgus osteotomy
NCT00900718	2006-2008 Conpleted	Comparison of Straumann Bone Ceramic and Bio-Oss in Combination With Guided Tissue Regeneration for Volume Preservation of Alveolar Ridge After Tooth Extraction	18-75 yrs	n.d.	Bone augmentation
NCT01147315	2009-2016 Completed	Prospective Study of Hybrid Bone Substitution With Calcium-phosphate Ceramic Biomaterial and Autologous Bone Marrow	18-75 yrs	n.d.	Hybrid bone substitution

		for Mandibular Osteoradionecrosis Treatment			
NCT01813188	2011-2014 Completed (Phase 2)	Non-inferiority and lower morbidity of the use of bone marrow mononuclear cells seeded onto a porous matrix of calcium phosphate, for the consolidation of tibial bone defects (pseudoarthrosis), compared with autologous bone graft	18-75 yrs	6 mths	Autologous bone marrow cells seeded onto a porous tricalcium phosphate ceramic and demineralized bone matrix
NCT01282034	2011-2016 Completed	Multicenter Randomized Controlled Trial for the Treatment of Knee Chondral and Osteochondral Lesions: Marrow Stimulation Techniques vs MaioRegen	18-60 yrs	24 mths	Marrow stimulation - Drilling or Microfractures
NCT01824706	2012-2016 Completed	A Prospective, Multicenter Observational Study Evaluating the Long Term Safety in Terms of Explantation Rate and Number of Infections of the Custom-made Bioceramic Implant CustomBone™	Child, adult, and senior	2 yrs	Craniectomy
NCT02389569	2016 Completed	Clinical Study of Biosilicate Under Resin Composite Restorations in Caries Affected Teeth	18-45 yrs	18 mths	Dental caries
NCT00841152	2009-2018	Comparison of two synthetic ceramic bone graft substitutes, bioactive glass and beta-tricalcium phosphate, in filling of contained bone defects following surgical evacuation of benign bone tumor or tumor-like conditions.	Adult and senior	12 mths	Hand and long-bone defects filling

NCT01742260	2013-2017 Phase 1	A Pilot Study to Demonstrate Safety and Feasibility of Cranial Reconstruction Using Mesenchymal Stromal Cells and Resorbable Biomaterials	18-80 yrs	n.d.	Repair of cranial defects by tissue engineering
NCT01771302	2013-2015	Efficiency of plasma rich in growth factors in combination with bone grafts in the healing of bone and soft tissues in lateral sinus floor elevation	Adult and senior	6 mths	Bone healing
NCT02910232	2014-2016 Phase 3	In Vivo Clinical Trial of Porous Starch - Hydroxyapatite Composite Biomaterials for Bone Regeneration	20-60 yrs	6 mths	Bone void filler of foot fracture
NCT01974362	2016-2017	Monolithic Zirconia Full-Mouth Implant Supported Rehabilitation Behavior	Adult and senior	12 mths	Place dental implants in both jaws (maxilla and mandible) according to manufacturer specifications

n.d.: not defined

4.5 CONCLUDING REMARKS AND FUTURE OUTLOOK

Bioceramics have been used very successfully within the human body by repairing and regenerating bone faster that would not restore by other means. These biomaterials are commonly used in orthopedic and dental surgery, but they are potentially suitable for a wide range of essential TE applications. TE has much to bring in respect to combining biomaterials, growth factors/bioactive molecules and cells. Innovative strategies present some of the current challenges in the field, and may constitute major breakthroughs in the future. Bioceramics offer desirable characteristics such as biocompatibility, chemical inertness in biological mediums and hardness, but they have low resistance to traction. Ongoing research involves the chemistry, composition, and micro- and nanostructure of the materials to improve the mechanical integrity of the bioceramics upon implantation, and appropriate porosity for the cellular adhesion, proliferation and differentiation. Biomimetic strategies designed for TE scaffolding have been concentrated on

3D-based porous and dense scaffolds and fibres, and hydrogels. The latter ones are of particular interest due to their high water content, besides biodegradability and biocompatibility. Although there have been significant advances in engineer new tissues, future developments in order to achieve major improvements should be focused and turn them into a clinically viable strategy. Strategies should be devoted on the clear understanding of the bioceramics-tissue interactions, and hierarchical structure for long-term service, and the related mechanical strength, especially the fatigue limit under periodic external stress.

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