DALTON PERSPECTIVE

# Ceramics for medical applications

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**Ceramics, glasses and glass ceramics include a broad range of inorganic non-metallic compositions. The apatite and related calcium phosphates have been of remarkable interest to biologists, mineralogists, inorganic and industrial chemists for many years. Calcium phosphate biomaterials, mainly hydroxyapatite, find many clinical applications in the repair of bone defects, bone augmentation and coatings for metal implants. Certain compositions of ceramics, glasses, glass ceramics and composites have shown bone bonding behaviour. These materials have become known as bioactive ceramics. A common characteristic of bioactive ceramics is a time-dependent, kinetic modification of the surface that occurs upon implantation. The surface forms a biologically active carbonate hydroxyapatite layer that provides the bonding interface with living tissues. The aim of this perspective is to present an overview of the different types of ceramics available for medical applications, focused mainly on bioactive glasses.**

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## Introduction

Ceramic materials for medical applications are an interesting practical field in obtaining biomaterials for implant production and/or fixation. The main advance have been achieved in the developed countries, due to the need to provide clinical treatment to a large population of patients. The increase in life expectancy and the social obligation to provide a better quality of life have been crucial factors to this progress. As life expectancy increases, so does in a dramatic way the number of patients with osteoporosis problems. If we also take into account the increase in the automotive field, with the negative factor inherent to this social advance, such as the increase in car accidents, the number of bone problems is rising alarmingly. The search for potential solutions to bone tissue problems exerts a strong demand for materials capable of substituting or repairing bones.

The industry of biomaterials includes organisations and companies which design, produce and manufacture materials used in the Health and Biological Sciences. The biomaterials can be classified as *biomedical*, with an artificial origin (metals, ceramics, polymers) and *biological*, that is with a natural (vegetal, animal or human) origin (Fig. 1).

Ceramic materials for medical applications were introduced in a given scenario (the 1970s) where failures of the biomaterials in use then, such as steel, cobalt alloys and poly(methyl methacrylate), began to be detected. Such failures were due, among other reasons, to the encapsulation of these materials. Hence, attention was focused on ceramic materials in an attempt to find good bone integration features.[1] The chemical elements used in the production of bioceramic materials form just a small set when compared with the whole Periodic Table, as depicted in Fig. 2. Fig. 3 shows the main characteristics of ceramic materials, all of them favourable when considering their use in implants, with the exception of their

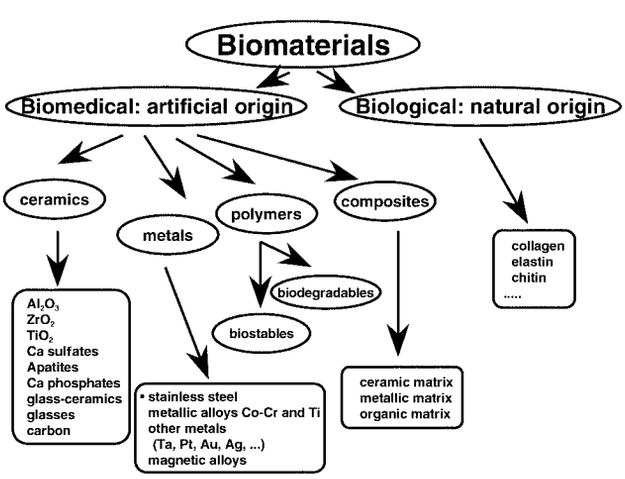


**Fig. 1** Classification of biomaterials.

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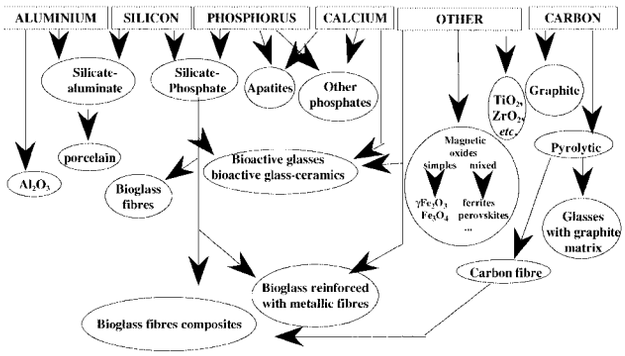



**Fig. 2** Chemical elements used in the production of bioceramic materials.

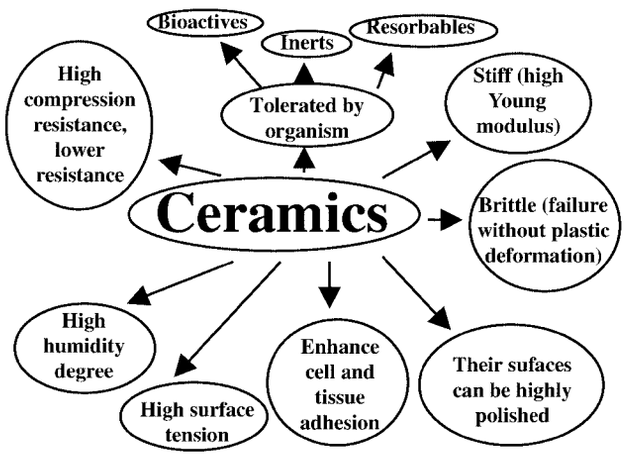



**Fig. 3** Main features of ceramic materials.

rigidity and fragility, two important drawbacks for such applications. The fragility of bioceramics has severely restricted their field of application, leaving aside $Al_2O_3$ and $ZrO_2$, which are being used for hip joint prostheses. Bioceramics, and phosphates in particular, could be used to manufacture ideal biomaterials, due to their high biocompatibility and bone integration, as well as being the materials most similar to the mineral component of the bones. One of the advantages that should be highlighted when analysing bioceramics is their low chemical reactivity, being almost totally inert and, therefore, biocompatible. However, not all bioceramics are chemically inert, and in fact, the ceramic materials used in reconstructive surgery can either be bioinert or bioactive. If we follow the definition of bioactivity proposed by Hulbert *et al.*,[2] a bioactive material is one that allows a specific biological response at its interface, enabling the formation of links between the tissues and said material. The bioactivity phenomena are fine examples of chemical reactivity. Like any other species, ceramic materials react chemically with their environment. However, the first ceramics used in medical applications, alumina ($Al_2O_3$) and zirconia ($ZrO_2$), are two types known as inert, and that was the main reason why they began to be employed in implant manufacturing.[3,4] To be precise, the dominant feature of these two materials is an extremely slow reaction kinetics, so that they should be considered as 'almost inert'. Obviously, other ceramics exhibit faster, or even very fast, reaction kinetics. As in any other chemical reaction, the reaction products of a substance with its environment may lead to an undesired result (*e.g.* corrosion of a metallic material), but it can also give rise to a favourable reaction product, through chemical transformation of the starting material to the desired final product. This is the case with bioactive ceramics; when in contact with physiological fluids, a chemical reaction towards the production of newly formed bone takes place. Many different factors affect the reactivity of any chemical substance and

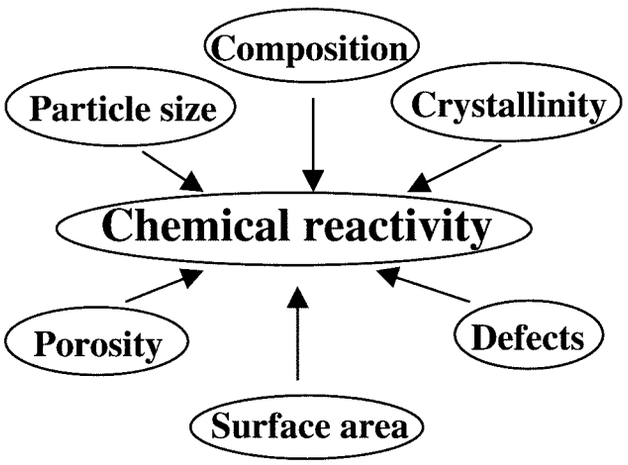



**Fig. 4** Some factors which have influence on the chemical reactivity of substances.

greatly determine its reaction kinetics. Fig. 4 shows some of these.

If we take into account the almost inert or bioactive nature of the different ceramics for medical applications, as well as kinetic factors such as particle size and porosity, three groups of bioceramics in use nowadays may be distinguished (see Fig. 5).

The final purpose of the artificial synthesis of ceramics for bone replacement (hard tissue) is to implant a ceramic material able to regenerate the damaged bone. This is feasible if the ceramic is bioactive. Otherwise, if the ceramic is inert, the bone will be replaced by a material that the organism can tolerate, but which cannot substitute it by means of bone regeneration.

The composition of the mineral component of bones, a non-stoichiometric carbonate hydroxyapatite, is shown in Fig. 6. According to the biomineralisation model present in nature, it was generally accepted that hard tissue formation starts from amorphous calcium phosphate which, after a series of heterogeneous equilibrium stages, reaches critical dimensions where the crystallisation of carbonate hydroxyapatite occurs. However, solid state $^{31}P$ NMR spectroscopy results indicate that the amorphous phase is never present in large amounts during bone development.[5] This technique also allowed the detection of acidic phosphate groups. Phosphate functions correspond to proteins with *O*-phosphoserine and *O*-phosphothreonine groups, which are probably used to link the inorganic mineral component and the organic matrix. Phosphoproteins are arranged in collagen fibres so that $Ca^{2+}$ can be linked at regular intervals which correspond to the inorganic crystal structure, giving rise to a repeatibility

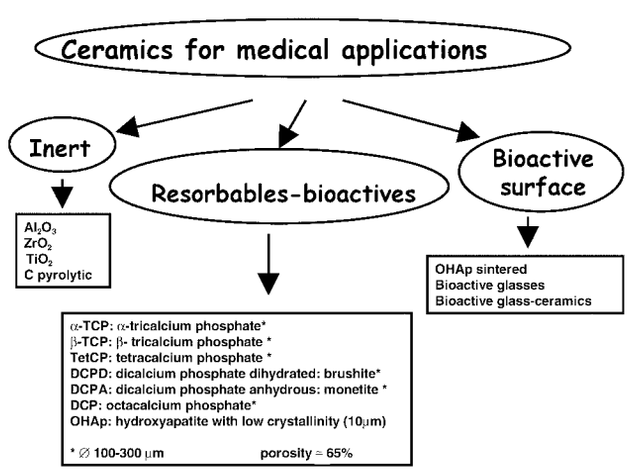



**Fig. 5** Types of bioceramics for medical applications.

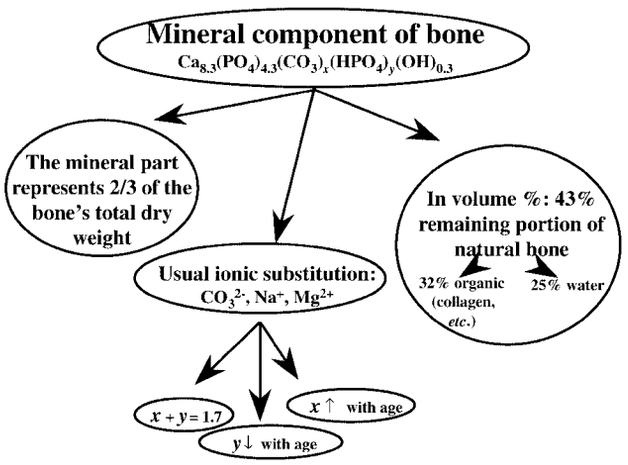



**Fig. 6** Composition of the mineral component of bones.

condition which enables the arranged sequence of a unit, *i.e.* the inorganic phase crystallinity. Besides, it is well known that natural bone is a nanocomposite material whose structure and mechanical properties stem from the arranged mineralisation of its components.[6] The search for bioactive ceramic materials brings us closer to the natural bone model, since the reactivity of these synthetic bioactive ceramics enables the growth, both *in vitro* and *in vivo*, of an apatite which is very similar to biological apatites, *i.e.* poorly crystallised, carbonated and non-stoichiometric with calcium deficiency.

The mechanical properties of ceramics are very poor, restricting their applications nowadays to non-load bearing implants, as is the case in ossicular surgery, or coatings of dental implants and metallic joints. The coating of a metallic material with ceramic is a complex process which greatly determines the clinical success, because the quality and endurance of the fixation at the interface largely depend on the purity, particle size, chemical composition of the coating, thickness of the coating layer and surface morphology of the substrate. An additional benefit obtained when coating a metallic implant with ceramic material is that the ion release from the metallic alloy is greatly reduced. The ceramic acts as an efficient barrier that retards the diffusion kinetics of metal ions towards the living body.

At present the applications of calcium phosphate ceramics are mainly focused on bone defect filling, both in dental and orthopaedic surgery. Hydroxyapatite is also being used to improve the bonding of hip joint prostheses, due to its outstanding biological properties such us atoxicity, lack of inflammatory response and absence of fibrous or immunological reactions. Therefore, the filling of bone defects and coating of metallic implants are the two main applications of ceramics used in manufacturing biomaterials. More recent applications are the production of calcium phosphate based cements, preparation of biphasic mixtures in an attempt to obtain a mineral component of bone tissue as similar as possible to biological apatites, and manufacturing cellular carrying substrates and biochemical factors for tissue engineering.

At present, donor tissue is predominantly used for bone replacements, both as alograft or autograft, although the percentage of use of synthetic materials is increasing. The value estimation of today's global market for such products is more than $1 Billion, with an annual growth rate of 7.7%. As the number of complex prosthetic revision surgery interventions increases, with the subsequent increase in use of bone filling material, it seems reasonable to forecast that this market will exceed a value of $2 Billion during the next decade. It is generally believed that this is one of the fields in orthopaedics not fully developed yet. Donor tissues exhibit good biocompatibility but also bring clear disadvantages, such as high costs, scarce availability, risk of disease transmission, *etc.*, when compared with synthetic materials. In the long run, the artificially produced materials will conquer a large share of this market, which can be divided into two different sectors, namely small and large bone defects.

A growing need exists in the fields of orthopaedic and dental surgery for a material able to improve the initial bonding of implants. Besides, it is also necessary to find a material or technique to reconstruct or augment bone defects. The synthetic materials available today present serious problems for these applications. Bone cements of poly(methyl methacrylate) (PMMA), for instance, do not attach to the bone. Besides, bone resorption phenomena take place around the cement, and the PMMA layer may crack. Moreover, the local rise in temperature when the monomer cross-links to form the polymer is sufficient to kill bone cells to a depth of nearly a millimetre.[7] On the other hand, granular calcium phosphate ceramic materials have been described as likely to induce a favourable physiological response. However, this material is difficult to apply, does not stay in its initial position and is not always resorbed.[8]

## Calcium phosphates

I shall include in this group not only the phosphates (crystalline solid materials), but also cements and biphasic mixtures with calcium phosphate content. The most used calcium phosphate in implant production is hydroxyapatite, because it is the most similar material to the mineral component of bones. However, and although it exhibits valuable properties as a biomaterial, biocompatibility, bioactivity, osteoconductivity, direct bonding with bones, *etc.*, its mechanical properties are poor. Basically, its fracture toughness is very low, severely restricting its field of application in orthopaedics. However, it is an excellent candidate for coating metallic prostheses or filling small bone defects. In the former application it would result in combination of the good mechanical properties of metals and alloys with the excellent biocompatibility and bioactivity of hydroxyapatite. In the latter application the filling of small bone defects of approximately 0.5 cm in diameter must be performed using osteoconductive materials. Such materials guide the bone growth and bond firmly from a mechanical point of view. The synthetic materials which exhibit such features are based in ceramic technologies, whether in simple or composite form. Apatites are among the best known and used ceramics for this application.

Before analysing the different possibilities that these ceramics might allow when chosen for implant production, it is worth studying the material to be substituted, *i.e.* the biological apatites. When compared against stoichiometric hydroxyapatite, the biological apatites, bone, dentine, enamel, exhibit a wide compositional range in their three sublattices. They are, therefore, markedly non-stoichiometric phases, always calcium deficient and with carbonate in their structure, with low crystallinity, large amounts of lattice defects and, due to their small particle size, high specific surface area. From a compositional point of view, the biological apatites are always calcium deficient and carbonated, being then carbonate hydroxyapatites. The apatite crystals which form the natural bones are smaller than 500 Å in size. This is a crucial factor in order to understand the solubility of biological apatites and the continuous bone regeneration due to constant dissolution–crystallisation cycles. The presence of $CO_3^{2-}$ in their structure is another fact of paramount importance;[9–11] it is the main source of lattice distortion, creating microstresses and crystalline defects in its vicinity which, in turn, play a fundamental role in the solubility. As a consequence, synthetic apatites aimed at emulating the biological scenario should exhibit small particle sizes and the presence of $CO_3^{2-}$.

These biological apatites (mineral component of the bones) are difficult to synthesize in the laboratory with carbonate contents equivalent to those in the bone. Although the

**Table 1** Calcium phosphates arranged by Ca : P ratio

| Name | Abbreviation | Formula | Ca : P |
|---|---|---|---|
| Tetracalcium phosphate | TetCP | $Ca_4O(PO_4)_2$ | 2.0 |
| Hydroxyapatite | OHAp | $Ca_{10}(PO_4)_6(OH)_2$ | 1.67 |
| Amorphous calcium phosphate | ACP | $Ca_{10-x}H_{2x}(PO_4)_6(OH)_2$ | |
| Tricalcium phosphate (α, β, γ) | TCP | $Ca_3(PO_4)_2$ | 1.50 |
| Octacalcium phosphate | OCP | $Ca_8H_2(PO_4)_6{\cdot}5H_2O$ | 1.33 |
| Dicalcium phosphate dihydrate (brushite) | DCPD | $CaHPO_4{\cdot}2H_2O$ | 1.0 |
| Dicalcium phosphate (monetite) | DCP | $CaHPO_4$ | 1.0 |
| Calcium phosphate (α, β, γ) | CPP | $Ca_2P_2O_7$ | 1.0 |
| Calcium pyrophosphate dihydrate | CPPD | $Ca_2P_2O_7{\cdot}2H_2O$ | 1.0 |
| Heptacalcium phosphate | HCP | $Ca_7(P_5O_{16})_2$ | 0.7 |
| Tetracalcium phosphate diacid | TDHP | $Ca_4H_2P_6O_{20}$ | 0.67 |
| Calcium phosphate monohydrate | MCPM | $Ca(H_2PO_4)_2{\cdot}H_2O$ | 0.5 |
| Calcium metaphosphate (α, β, γ) | CMP | $Ca(PO_3)_2$ | 0.5 |

carbonate inclusion in itself is very simple (in fact, when producing stoichiometric apatites in the laboratory, strict control of the synthesis conditions is needed to avoid carbonate inclusion), the carbonate content is always different from the fraction of carbonates in natural bone (4–8% w/w)[11] and/or located in different lattice positions.[10] The carbonate easily enters into the apatite structure, but the problem lies in the amount that should be introduced taking into account the carbonate content of biological apatites. When the aim is to obtain carbonate hydroxyapatite and the reaction takes place at high temperatures, the carbonates enter and occupy lattice positions in the $OH^-$ sublattice (A type apatites). In contrast, the carbonates in biological apatites always occupy positions in the $PO_4^{3-}$ sublattice (that is they are B type apatites).[10] In order to solve this problem, low temperature synthesis routes have to be followed, allowing one to obtain carbonate hydroxyapatites with carbonates in phosphate positions.[11] However, the amount entered remains to be solved, and usually is lower than the carbonate content of the mineral component of bones.

These calcium deficient and carbonated apatites have been obtained in the laboratory by various techniques; nowadays, it is known that apatites with low crystallinity, calcium deficiency and carbonate content can be obtained, but with carbonate contents usually unequal to those of natural bones.[12–15] Therefore, the main problem remains in the control of carbonate content and lattice positioning.

In the wide range of existing or potentially viable calcium phosphate phases it is important to understand the close relationship between Ca : P ratio, acidity and solubility. The lower the Ca : P ratio is, the higher are the acidity and solubility of the mixture. For ratios below 1 : 1 both acidity and solubility are very high, and these two parameters decrease substantially for ratios close to 1.67 : 1. The Ca : P ratio is a very useful parameter for the scientist working in this field. Table 1 shows several calcium phosphates arranged according to their Ca : P ratio.

On the other hand, in the literature on phosphates focused on calcium phosphate cements, the technique employed for obtaining such cements is to mix the different components; one of them is responsible for curing the mixture. For instance, in Constanz cement,[16] the first of its kind to be commercialised, the final product is a carbonate apatite (dahlite) with low crystallinity and a carbonate content reaching 4.6%, substituting the phosphate groups (B type carbonateapatite), as is the case in bones. Constanz cement is obtained from a dry mixture of α-tricalcium phosphate, α-$Ca_3(PO_4)_2$, calcium phosphate monohydrate, $Ca(H_2PO_4){\cdot}H_2O$ and calcium carbonate, $CaCO_3$. The Ca : P ratio of the first component is 1.50 : 1, and 0.5 : 1 for the second one, both values significantly lower than 1.67 : 1 for hydroxyapatite. A liquid component, a sodium monoacid phosphate solution, is then added to this solid mixture, which allows the formation of an easily injectable paste that will cure over time. The paste curing happens after a very reasonable period of time when considering its use in surgery. In fact, after five minutes it shows a consistency suitable for injection, and upon ten minutes is solid without any exothermal response, exhibiting an initial resistance of 10 MPa. 12 hours later, 90% of its weight has evolved to dahlite, with a resistance under compression of 55 MPa, and 2.1 MPa when under stress. This cement is then resorbed and gradually replaced by newly formed bone.

Calcium phosphate cements which can be resorbed and injected are being commercialised by various international corporations, with slight differences in their compositions and/or preparation, and research is still underway in order to improve the deficiencies still present.[17–19] These cements cure in field, are very compatible with bone and seem to resorb slowly; during this gradual process the newly formed bone grows and replaces the cement. However, their properties are still insufficient for reliable application. There are problems related to their mechanical toughness, the curing time, application technique on the osseous defect and final biological properties. New improvements in the development of these new cements have been described recently, solving at least in part some of these disadvantages. For instance, the curing time has been shortened, even in contact with blood, and the toughness under compression has also improved.[20]

Several attempts have been made to synthesize the mineral component of bones starting from biphasic mixtures of calcium phosphates.[21,22] Hence, bone replacing materials based on mixtures of hydroxyapatite and α-TCP have been prepared; under physiological conditions such mixtures evolve to carbonate hydroxyapatite. The chemical reactions are based on equilibrium conditions between the more stable phase, hydroxyapatite, and the phase prone to resorption, α-TCP. As a consequence, the mixture is gradually dissolved in the human body, acting as a stem for newly formed bone and releasing $Ca^{2+}$ and $PO_4^{3-}$ to the local environment. This material can be injected, used as a coating or in any other form suitable for application as bulk bone replacement, forming of bulk pieces or filling of bone defects.[23] At present, a wide range of biphasic mixtures are under preparation, using various calcium phosphates, bioactive glasses, calcium sulfates, *etc*.

The promising results obtained with cements and biphasic mixtures seem to indicate that it is easier to obtain precursors of synthetic apatites that, when in contact with the biological environment, can evolve towards similar compositions to that of the biological apatite, than to obtain apatites in the laboratory with similar compositional and structural characteristics to those of the biological material, and in adequate quantities, *i.e.* large, industry-scale amounts with precise composition and easily repeatable batch after batch, for use in the production of ceramic biomaterials.

Bioceramics aimed at the replacement or filling of bones could be obtained by synthesis of apatite precursors through different calcium phosphate mixtures, using a wet route. If the

information gathered from the calcium cements is put to use, it would be necessary to eliminate the solution added to cure the mixture and search for compositions and ratios that allow one to obtain precursors that, when in contact with the body fluids, evolve chemically towards the formation of carbonate hydroxyapatite crystals, with small particle size and low crystallinity, calcium deficient and with a carbonate content of approximately 4.5% w/w, located in the $PO_4^{3-}$ sublattice.

Traditionally, the calcium phosphate ceramics have been processed by high temperature treatments.[24,25] If the products have previously been synthesized by the wet route at low temperatures, this leads to a very crystalline material, and therefore not similar to biological apatites. Owing to the thermal decomposition of most calcium phosphates at high temperature, this type of process is restricted basically to obtaining stoichiometric hydroxyapatite and tricalcium phosphate. It can be employed in the production of both dense and porous pieces. The latter allow the growth of natural tissue inside the pores, helping mechanically to stabilise the implants. Although these treatments are still in use nowadays,[26,27] they reduce significantly the reactivity of the ceramics and the growth kinetics of the bone. Therefore, new forming methods at lower temperatures have been developed,[28–30] allowing one to obtain pieces without altering the crystallinity of the ceramic starting material. The combination of the synthesis of calcium deficient, low crystallinity carbonate hydroxyapatites with processing methods that preserve their chemical and microstructural features is an excellent alternative to the production of bioceramic pieces.

If materials in powder form instead of pieces are used for bone filling applications, the main advantage is their superior adaptability to the profile of each defect, while clinical insertion is not so adequate. Such materials are difficult to place and secure in the implant region, and their particles retain the potential to migrate for weeks or even months. To avoid this drawback the powder is mixed with a degradable carrier matrix. At present the trend is to obtain materials suitable for injection.[31] Both organic and inorganic matrices can be used. Among the organic matrices, non-absorbable polymers such as PMMA,[32,33] polyethylene (PE)[34] and polysulfone are being used, but the main problem is that such matrices reduce the bioactivity of the implant. Biodegradable polymers are also in use, such as polylactic acid (PLA)[35] and polyglycolic acid (PGA), as well as natural polymers[36,37] (collagen, cellulose and starch). An inorganic matrix also in use is calcium sulfate (Hapset).[38]

## Bioactive glasses

In the range of ceramic materials, and according to their microstructure, bioactive glasses (amorphous solid materials) are placed at the farthest end from the conventional ceramics (crystalline solid materials). The glasses might be defined as solid materials with enormous structural disorder, or as liquid materials with large viscosity values. As a concept the latter definition is more adequate. However, the governing difference between a crystalline ceramic and a glass is the order–disorder balance in their respective lattices. We can find the same chemical composition forming an ordered or disordered structural lattice. Fig. 7 shows an illustrative example, which is in turn the cornerstone of bioactive glasses: silica ($SiO_2$).

Certain compositions of glasses and glass ceramics (amorphous solid materials with crystallisation nuclei) containing $SiO_2$ and CaO are bioactive as the surfaces of these materials give rise to the formation of a silica hydrogel layer that allows subsequent crystallisation of the apatite-like phase.[39]

Melting and sol–gel are two well known methods for producing glasses. Figs. 8(a) and 8(b) summarise the stages of

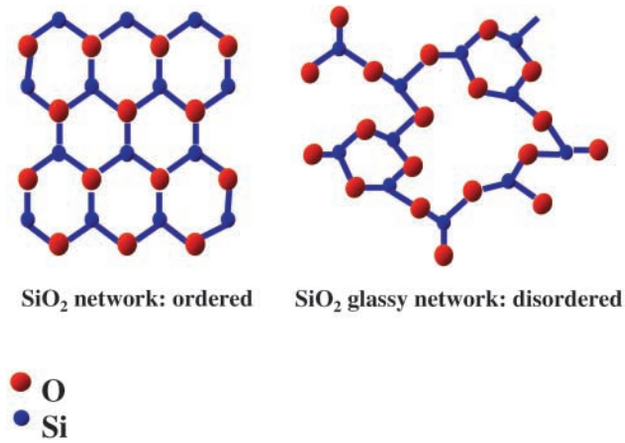



**Fig. 7** Two very different structural situations for an identical chemical composition, $SiO_2$, namely ordered and disordered structure, thus giving rise to a crystalline ceramic and a glass, respectively.

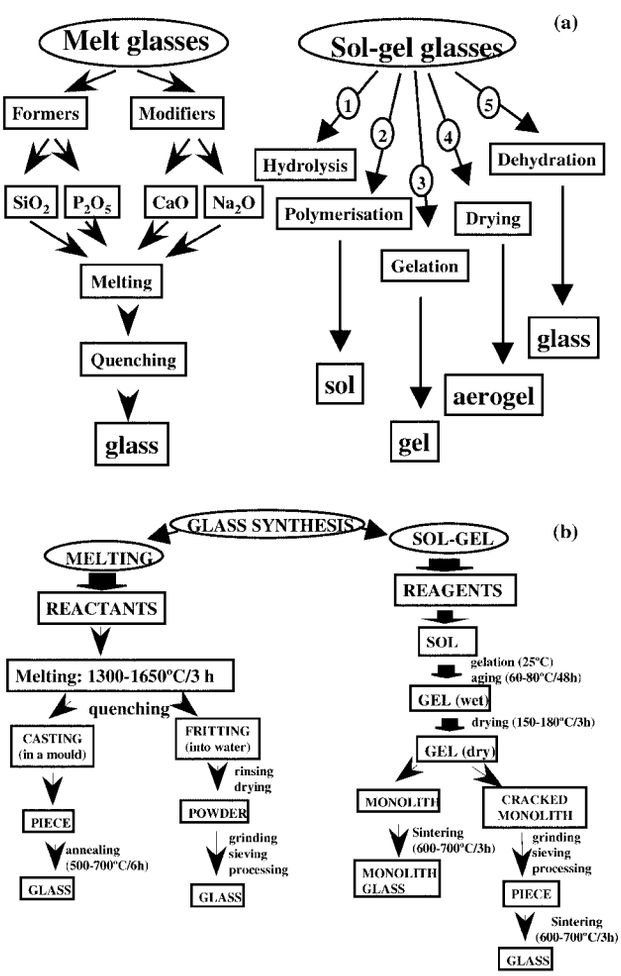



**Fig. 8** (a) Schematic diagram of the main stages in melting and sol–gel processes. (b) Stages in melting and sol–gel processes, with temperature range and duration.

both processes, including the corresponding working temperatures when the aim is to obtain bioglasses. The purpose of the network formers is to build Si–O–Si bonds, while the network modifiers try to break apart those bonds, thus allowing the melt to solidify with a high degree of disorder. Its presence caters for lower melting temperatures and viscosity values, reducing the economic costs of glass production, while ensuring a high degree of disorder. Fig. 9 shows the disordered structure of a glass in the $SiO_2$–CaO–$Na_2O$ system. This disordered structure, enhanced by the presence of lattice modifiers, results in high reactivity in an aqueous environment of these glasses.

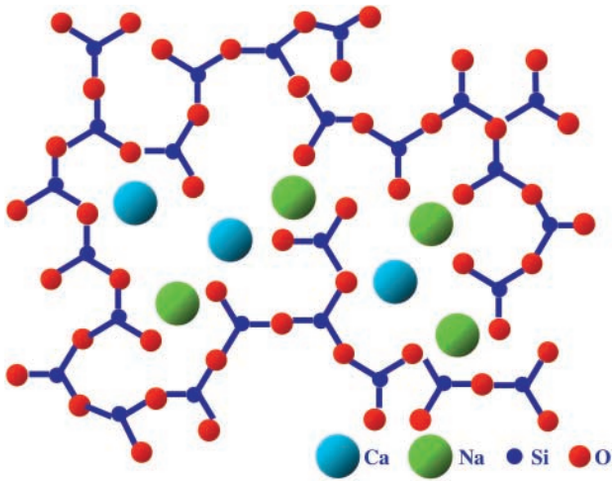



**Fig. 9** Disordered structure of a glass in the $SiO_2$–CaO–$Na_2O$ system.

This high reactivity is in fact the main advantage for their application in periodontal repair and bone augmentation, because the reaction products obtained from these types of glasses and the physiological fluids lead to crystallisation of the apatite-like phase.

Taking into account the main factors that affect the chemical reactivity of solids, depicted in Fig. 4, we shall now address the chemical reactivity of bioactive glasses.

### Composition

The first bioactive glass, in the system $Na_2O$–CaO–$P_2O_5$–$SiO_2$, was reported in 1971 by Hench *et al.*[40] The synthesis process consisted of melting the precursor mixture and quenching. In the following years several compositions contained in the phase equilibrium diagram of such a system were studied.[41–44] The analysis of all results obtained allowed this phase diagram to be divided into areas which corresponded to bioactive glasses, non-bioactive glasses, soluble or resorbable glasses, or where no glasses were obtained.[45]

New components were added to the system almost simultaneously in order to act as network formers and/or modifiers and to decrease the temperature of obtaining bioglasses. However, the main purpose of their inclusion was to improve the properties focused on clinical applications, *i.e.* to increase the bioreactivity or at least to preserve or increase the bioactivity, while adding new properties to the materials. In this sense, the addition of $K_2O$, MgO, $CaF_2$, $Al_2O_3$, $B_2O_3$ or $Fe_2O_3$ was tested.[46] However, all this effort did not always lead to positive results, since the addition of some of these oxides degraded or totally destroyed the bioactive behaviour of the glasses. For instance, 3% $Al_2O_3$ added to the initial composition of Hench, in order to improve its mechanical properties,[47] eliminated its bioactivity, and addition of $Fe_2O_3$ to obtain glass ceramics for hyperthermia treatment of cancer[48] decreased the bioactivity. In 1997, Brink *et al.*[49,50] studied the *in vivo* bone-bonding ability of 26 melt glasses in the system $Na_2O$–$K_2O$–CaO–MgO–$B_2O_3$–$P_2O_5$–$SiO_2$, concluding that the compositional limits for bioactivity were: 14–30 mol% of alkali metal oxides ($Na_2O$ + $K_2O$), 14–30 mol% of alkaline earth metal oxides (CaO + MgO), and less than 59 mol% of $SiO_2$.

The alternative approach to the $Na_2O$–CaO–$P_2O_5$–$SiO_2$ system was to simplify it instead of turning it more complex. The use of the sol–gel technique to prepare glasses helped in this way. Sol–gel allows one to leave out $Na_2O$ as fusing agent and to work with the ternary system CaO–$P_2O_5$–$SiO_2$. It also allows one to obtain high purity glasses, more homogeneous than those obtained by melting, requiring only low processing temperatures. This method makes it possible to expand the bioactive compositional range studied in the phase equilibrium diagram of melted glasses, and the glasses so obtained exhibit higher surface area and porosity values, critical factors in their bioactivity.[51–55]

Therefore, the application of the sol–gel synthesis technique led to a simplification of the system. The first oxide to be left out was $Na_2O$. Some examples of bioactive glasses in the ternary CaO–$P_2O_5$–$SiO_2$ system are given in references.[51,56–60] For illustrative purposes, there is a description of the preparation of a bioactive glass with composition (in mol%) $SiO_2$(70), CaO(26), $P_2O_5$(4).[59] The glass was prepared by hydrolysis and polycondensation of 25 mL of tetraethyl orthosilicate (TEOS), 2.15 mL of triethyl phosphate (TEP), and 9.73 g of $Ca(NO_3)_2 \cdot 4H_2O$, in stoichiometric amounts to obtain a nominal composition (mol%) of $SiO_2$(70), CaO(26), and $P_2O_5$(4); 1 M $HNO_3$ was used to catalyse the TEOS and TEP hydrolysis, using a molar ratio of ($HNO_3$ + $H_2O$) : (TEOS + TEP) = 8 : 1. Each of the reactants was consecutively added in 1 hour intervals under continuous stirring. Next, the solution was introduced in a hermetically sealed cylindrical Teflon® container where it was allowed to gel at room temperature and then aged at 70 °C for 3 days. The drying was carried out at 150 °C for 52 h, having replaced the previous container lid with one featuring a hole 1 mm in diameter.

The dried gel was ground and sieved. Grains (ranging in sizes from 32 to 63 μm) were selected. Fractions of 0.5 g were used to prepare disks (13 mm in diameter and 2 mm in height) by uniaxial pressure of 50 MPa for 5 min and then 150 MPa of isostatic pressure for 5 min. To determine the stabilisation temperature, thermogravimetric and differential thermal analyses (TG/DTA) of the dried gel were carried out using a Seiko Thermobalance TG/DTA 320. Based on the results that no further weight loss was recorded after treating the dried gel at or above 700 °C, the stabilisation treatment was carried out by heating the dried gel disks at 700 °C for 3 h. Fig. 10 shows the described process.

The next stage in the simplification of the ternary system was to eliminate another component. According to the information gathered from the study of other systems, $SiO_2$ seemed very important for the bioactive behaviour of the glass;[61] therefore, the obvious candidate for elimination was $P_2O_5$. This binary system was tested by Kokubo and co-workers,[62,63] producing glasses of the binary system $SiO_2$–CaO with a $SiO_2$ content less than or equal to 65%, prepared by melting; Vallet-Regí and co-workers also prepared glasses in this system, with $SiO_2$ contents of up to 90% (50–90% $SiO_2$), by the sol–gel technique.[60,64–66]

### Crystallinity and defects

Bioglasses are solid materials which can be classified as amorphous according to the results of X-ray diffraction studies; therefore, they exhibit a disordered lattice. Fig. 11

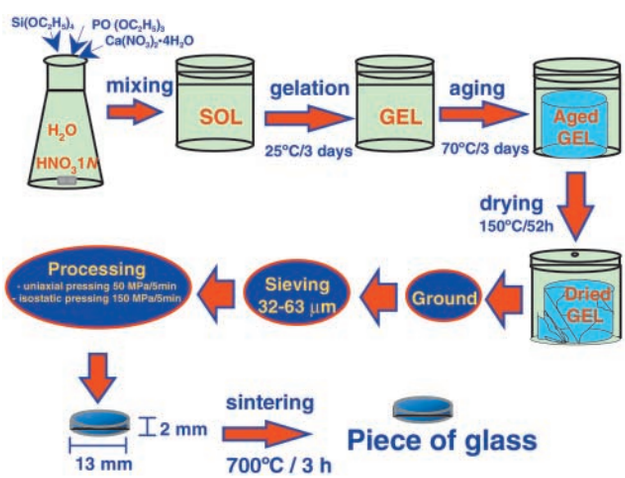



**Fig. 10** Description of the synthesis process for bioactive glasses.

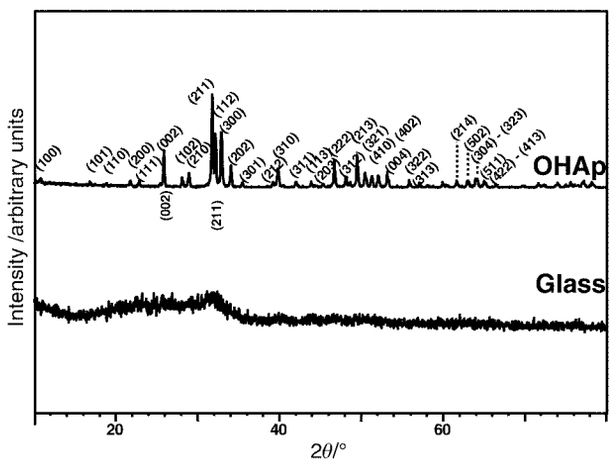



**Fig. 11** X-Ray diffraction patterns of a bioactive glass (amorphous material) and hydroxyapatite (OHAp, crystalline ceramic material).

shows the X-ray diffraction pattern of a glass with composition (in mol%): $SiO_2$ (80), $P_2O_5$ (4), CaO (16).[67] We can see the marked contrast between the diffraction pattern of hydroxyapatite a crystalline ceramic material which exhibits well defined diffraction maxima, and that of the glass, an amorphous ceramic material where no maxima are detected; only the characteristic amorphous band at low angles is visible. The inherent disorder of the glassy structure (high number of structural defects and non-crystallinity) can be related to the high reactivity of these materials when in contact with flowing media, as we shall see in the bioactivity studies.

### Textural properties: surface area, pore volume and pore size distribution

It is well known that the kinetics of surface reactions is closely related to the texture (porosity and surface area) of the materials. In the case of bioactive glasses this is a very important factor. Following again with an example, we may analyse the specific surface and pore volume of four glasses in the ternary system $SiO_2$–CaO–$P_2O_5$ obtained by a sol–gel method.[51–55] Table 2 includes the chemical composition of these glasses, as well as their respective values of BET (Brunauer–Emmett–Teller) surface and mesopore volume, data obtained by $N_2$ adsorption, while Figs. 12 and 13 show the volume of Hg intruded as a function of the nanopore diameter of the synthesized glasses and $N_2$ adsorption isotherms, respectively. It is worth noting that the minimum surface area of these glasses is in the order of 100 $m^2\ g^{-1}$, although surface values larger than twice such a minimum are detected for higher $SiO_2$ contents. On the other hand, we can observe an increase of the pore volume in the macropore range as the $SiO_2$ content decreases. On the other hand, the surface area increases with the $SiO_2$ content. The textural properties of the sol–gel glasses in the system $SiO_2$–CaO–$P_2O_5$ are clearly determined by the properties of the three glass components. A larger $SiO_2$ percentage leads to a lower macro- and meso-pore volume, and the pore diameter decreases in both ranges. However, the surface area follows the opposite trend.[52,54]

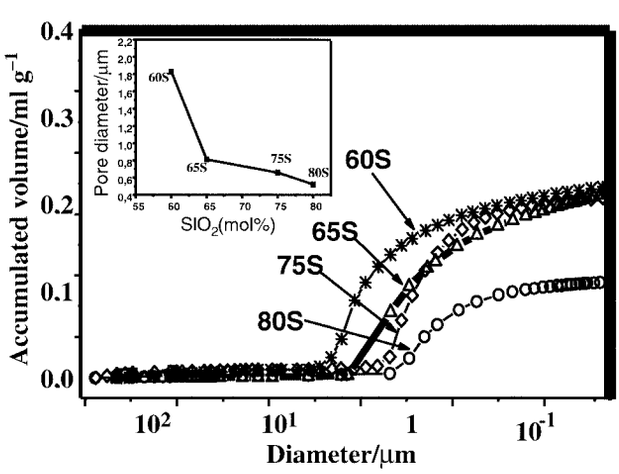



**Fig. 12** Volume of Hg intruded as a function of pore diameter of the synthesized glasses, with compositions detailed in Table 2.

**Table 2** Molar composition of the glasses, BET surface and mesopore volume data obtained by $N_2$ adsorption

| Glass | $SiO_2$ (mol%) | CaO (mol%) | $P_2O_5$ (mol%) | $S_{BET}$/ $m^2\ g^{-1}$ | Pore volume/ $cm^3\ g^{-1}$ |
|---|---|---|---|---|---|
| 60S | 60 | 36 | 4 | 95 | 0.35 |
| 65S | 65 | 31 | 4 | 125 | 0.32 |
| 75S | 75 | 21 | 4 | 175 | 0.21 |
| 80S | 80 | 16 | 4 | 222 | 0.24 |

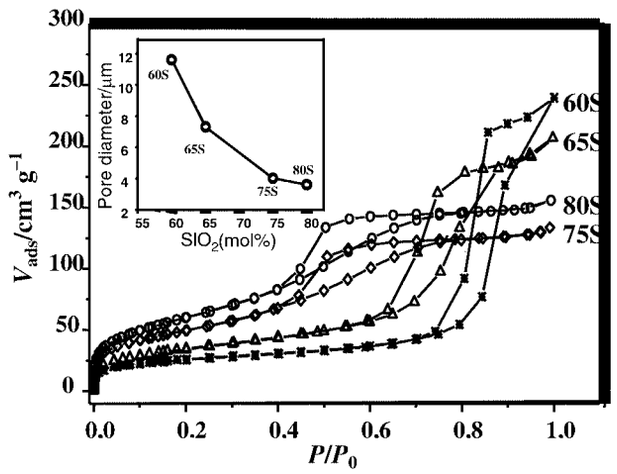



**Fig. 13** $N_2$ adsorption isotherms of the synthesized glasses, with compositions detailed in Table 2.

Although the influence of the texture of the substrate on the formation of apatite is generally admitted, the detailed nature of the nucleation process of the apatite is still a matter of debate. The practical totality of authors focuses the discussion on apatite nucleation upon the role of the silanol groups existing on the glass surface under the environmental conditions where the essays are conducted.[68–70] Wang and Chaki[71] show an epitaxic relationship between Si(111) and apatite in [102] orientation. Interestingly, the phosphorus and calcium of the substrate are generally considered as a mere reservoir that influences the supersaturation of the solution as they are leached from the glass. Nevertheless, phosphorus and calcium as components of bioactive glasses could in fact be potential nucleation centres for apatite crystallisation. To gain knowledge on the influence that the chemical nature of the glass surface exerts on the nucleation events leading to the formation of apatite, the group of Vallet-Regí showed the relationships between the *in vitro* bioactivity of $SiO_2$–CaO–$P_2O_5$–(MgO) sol–gel glasses and the textural properties and chemical nature of the glass surface.[54]

During the 1970s and 1980s, bioactive glasses were exclusively obtained by the traditional method of melting and slow cooling,[40] producing materials with very small specific surface area and porosity. The increase in bioactivity of the sol–gel glasses when compared to those obtained by the melting technique can be explained by the great differences in the textural properties of the material, which enact a strong influence on the reactivity.[53–55] The high specific surface area and porosity of sol–gel glasses determine the formation kinetics of the carbonate hydroxyapatite surface layer, formed on the glass surface as a product of the reaction between the glass and the surrounding flowing medium.

### Assessment of *in vitro* bioactivity

One of the main features of bioactive materials is the ability to form an apatite-like layer on their surfaces when in contact with physiological fluids *in vivo* or with simulated body fluids (SBF) *in vitro*.[41] This layer appears to be associated with bioreactivity, and it is speculated that it is responsible for the bonding of bioactive ceramics to the host bone.

In bioactive silica-based glasses and glass ceramics a high surface silica gel layer is formed by partial network dissolution and surface polycondensation reactions that occur previous to formation of the calcium phosphate-rich layer.[41] The silica gel and carbonate hydroxyapatite layers provide adsorption sites for the cellular growth factors generated by macrophages and stem cells, promoting bone formation. The recognition that the silica gel layer plays a role in carbonate hydroxyapatite nucleation and crystallisation led to the development of a family of bioactive glasses.[51,56,59,72]

The carbonate hydroxyapatite layer is also formed on the surface of bioactive materials when they are soaked in aqueous solutions that simulate different properties of human plasma, such as pH, ionic composition, *etc*. Thus, in recent years *in vitro* studies of the bioactivity of new implantation material candidates increasingly have been conducted. The *in vitro* bioactivity of glasses that release calcium and phosphorus ions into a solution has been studied by Hench and co-workers in a solution of tris(hydroxymethyl)aminomethane buffered with HCl at a pH of 7.25 (Tris buffer) at 37 °C.[73] In 1990 Kokubo *et al.* proposed a simulated body fluid,[74] an acellular aqueous solution with an ion concentration and pH almost equal to those of human blood plasma. Since SBF solution itself contains $Ca^{2+}$ and $HPO_4^{2-}$ ions, it can be used to study the *in vitro* bioactivity of a wide variety of materials.

In *in vitro* assays, when the bioactive glasses are in contact with any of these solutions, three phenomena occur: release of ions, pH modifications and growth of an apatite-like product layer on the glass surface. Therefore the assessment of the processes taking place should be based on monitoring and characterisation of the modifications in the fluid and at the glass surface. Fig. 14 indicates the type of analyses and the techniques that should be used in order to elucidate the process.

Some examples will again be used to explain the evolution of the reaction between a bioactive glass and its surrounding medium. First of all, in order to carry out the *in vitro* study, a medium has to be chosen. Then, a protocol will be established. The study can be performed on different aqueous biological fluids. Some of them include: Tris buffer,[73] SBF,[74] newborn bovine serum[75] and some cell culture media such as Dulbecco's modified Eagle's medium.[76] Regarding the protocol, the assay can be performed in a static medium,[47,59,60] renewed every so often,[77] renewed with continuous stirring[78] or in a dynamic medium where the solution is constantly exchanged with a peristaltic pump.[65]

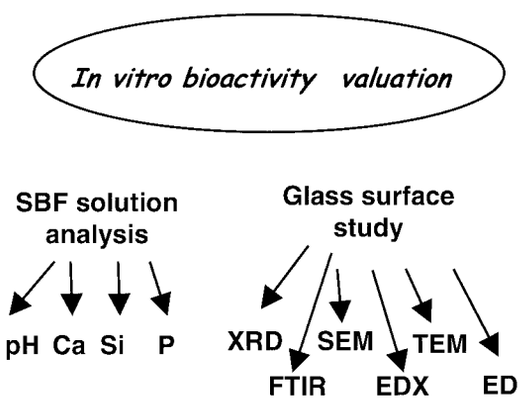
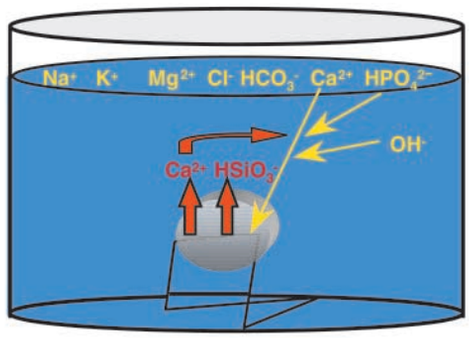



**Fig. 14** Techniques employed in characterisation of bioactive glasses.




Ionic composition of SBF and Human Blood Plasma (mM)

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

**Fig. 15** Chemical process taking place between a bioactive glass and a solution of SBF.

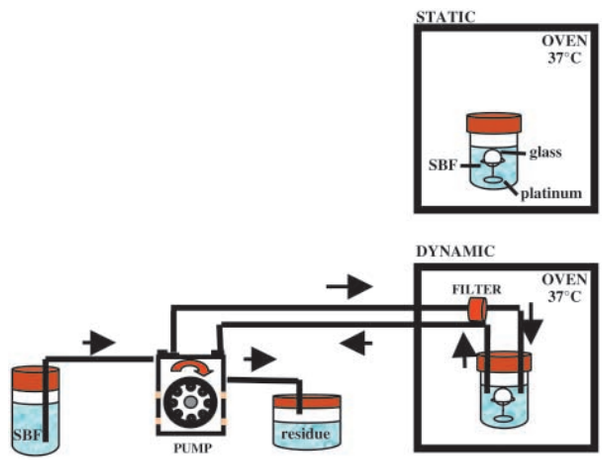



**Fig. 16** Devices needed to work according to static or dynamic protocols.

Fig. 15 shows the ionic exchange outline between the bioglass and the SBF, while Fig. 16 depicts the static and dynamic protocols.

**Formation of an apatite-like layer.** In the first place, the changes that the bioglass surface experiences can be verified by means of FTIR spectroscopy. Fig. 17(a) indicates the formation of an apatite-like layer on the glass surface after soaking in SBF. Silicate absorption bands at about 1085, 606 and 462 $cm^{-1}$ were observed in the glass spectra before soaking. Phosphate absorption bands at about 1043, 963, 603, 566 and 469 $cm^{-1}$ and carbonate absorption bands at approximately 1490, 1423 and 874 $cm^{-1}$ were observed in the spectra of materials scraped from the surfaces of soaked glass disks. An increase in the intensity of the carbonate bands, observed in the spectra of glasses, was associated with the soaking period in SBF solution. The phosphate and carbonate absorption bands observed on the glass surfaces after soaking were similar to those for synthetic carbonate hydroxyapatite.[59] The existence of these bands in the spectra of materials formed on the glass surface not only confirms the formation of an apatite-like layer, but also indicates that the apatite-like layer material is a carbonate hydroxyapatite similar to biological apatites, in which a coupled substitution of $Na^+$ by $Ca^{2+}$ and $CO_3^{2-}$ by $PO_4^{3-}$ is observed.[79,80]

The changes in the bioglass surface can also be monitored by X-ray diffraction. Given the amorphous nature of the glass, and its evolution towards an apatite of very low crystallinity, at first it does not seem a very adequate technique for such study. However, it is a very useful tool to visualise the transformations

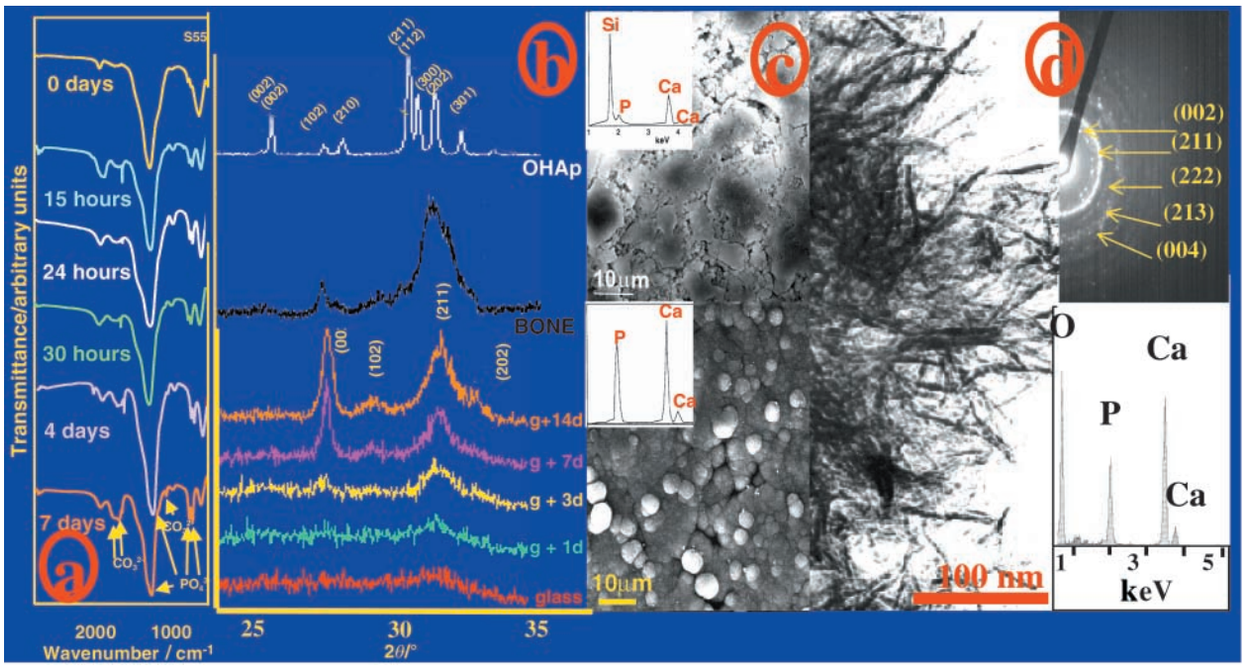



**Fig. 17** The modifications of the bioactive glass surface can be identified by (a) FTIR, (b) X-ray diffraction, (c) SEM, EDS (energy dispersive spectroscopy), (d) TEM, ED and EDS.

on the glass when in contact with SBF, following the evolution with soaking time. It also allows one to compare the diffraction patterns obtained with those of natural bone; for soaking times equal to or above seven days, clear similarities can be observed. For comparative purposes, a standard diffraction pattern of hydroxyapatite (PDF# 09-0432) has been included in Fig. 17. As can be observed, the diffraction patterns of bioactive glasses show two diffuse reflections centred at $2\theta$ values of 26 and 32° that correspond to the hydroxyapatite (002) and (211) reflections, respectively. Even after 7 days of soaking in SBF the XRD patterns correspond to an amorphous material. Therefore, the technique does not detect the presence of a crystalline phase on the glass surface.

The changes at the bioglass surface can clearly be observed by scanning electron microscopy (SEM) techniques. Thus, Fig. 17(c) shows images of the glass surface, before and after soaking in SBF for one week. These images allow one to confirm the formation of a layer constituted by spherical particles, which coats the whole surface of the initial glass. An additional image at a higher magnification (Fig. 18) shows the morphology of such spherical particles. We can observe that the particles are formed by several hundred small crystalline aggregates. The combination of SEM and EDS techniques yields additional information about the nature of this newly formed layer. The EDS profiles of the glass surface before and after soaking in SBF indicate at first the presence of Si, P and Ca in relative concentrations similar to the percentages of the glass under assay, while the profile taken after 1 week of soaking in SBF reveals the presence of P and Ca only, with a Ca : P ratio of approximately 1.25 : 1. These results support the growth of a layer with similar composition to that of biological apatites.

In turn, the particles observed by SEM can further be studied by TEM and ED, analysing their composition by means of EDS equipment connected to the TEM microscope. Fig. 17(d) shows the high magnification image, ED pattern and EDS spectrum of particles at the apatite like layer grown onto the bioglass surface upon 1 week of soaking in SBF. A small area was selected using the microdiffraction technique. The ED pattern obtained showed the presence of diffuse diffraction rings in which the interplanar spacings agreed with those of an apatite-like structure, leading us to believe that crystalline nuclei were embedded in a glassy matrix. In the corresponding micrograph the needle-like shape of the aggregated crystals forming the spherical particles may be observed. Taking into account the hydroxyapatite lattice parameters ($a = 9.5$ and $c = 6.8$ Å), and its symmetry (hexagonal, space group $P6_3/m$) (Fig. 19), most likely its unit cells will be arranged along the $c$ axis. This would justify a preferred orientation that gives rise to an oriented growth along the $c$ axis and a needle-like morphology, which agrees with the morphology observed by TEM. On the other hand, the EDS spectrum obtained with a TEM microscope showed that the crystals were composed of Ca, P and O, corresponding to biological apatites.

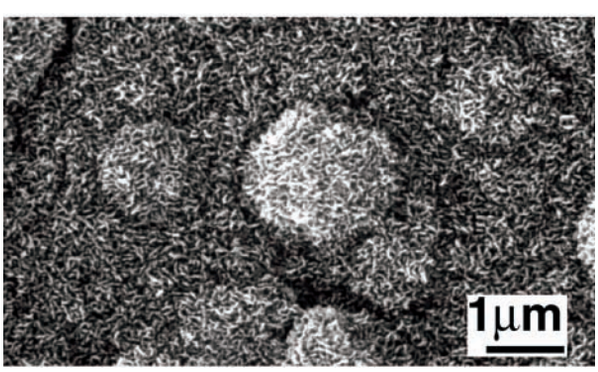



**Fig. 18** Micrograph of a bioactive glass after soaking in SBF for one week.

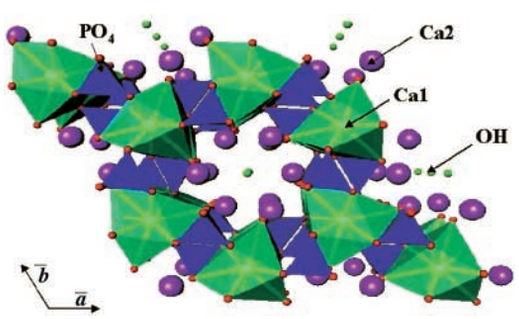



**Fig. 19** Structure of the hydroxyapatite (hexagonal, space group $P6_3/m$).

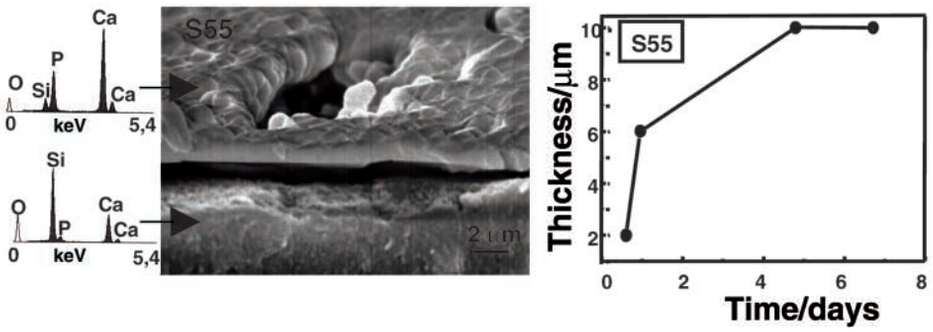



**Fig. 20** Evolution with time of the apatite-like layer thickness on a bioactive glass. SEM and EDS techniques have been used.

Another interesting aspect of the apatite-like layer is its thickness. The combination of SEM and EDS techniques can be very useful in this respect. In Fig. 20 the cross section of 55S glass (55, $SiO_2$ percentage; S, sol–gel) after 15 hours of soaking is shown. The EDS spectra inside the glass and on the layer are also included. As observed, the obtained analysis of the inner region agrees with the nominal glass composition, that is 55% $SiO_2$–41% CaO–4% $P_2O_5$ (in mol%). However, in the EDS spectrum of the layer, a remarkable increase in the concentrations of Ca and P, together with a significant decrease of Si was observed. This indicates the formation of an apatite-like material. On the other hand, the SEM study of the cross section of samples after different soaking times allowed monitoring of the evolution of the layer thickness with the soaking time in SBF. The layer thickness grew from 2 μm after 15 h of immersion to 10 μm after 5 days of assay. There is no difference in layer thickness between 5 and 7 days, which suggests that, at least *in vitro*, the apatite-like layer does not keep growing indefinitely.

**Changes in SBF composition.** The behaviour of all bioglasses soaked in SBF follows a general trend. However, we shall consider in this section a bioactive glass in the Ca–Si system which, since no phosphorus content is included, will simplify the scenario. The glass selected has a nominal composition (in mol%) 80 $SiO_2$, 20 CaO (80S20C).[60,64,65,81]

In Fig. 21 are shown the variations with time of pH and the concentrations of calcium, phosphorus and silicon in solution for 80S20C glass when a static protocol was used and also when a dynamic protocol, in which the solution was renewed at a rate of 1 mL $min^{-1}$, was used. Every point was obtained as an average of two values. For comparison, the values in the human plasma are included in the figure. In static protocol

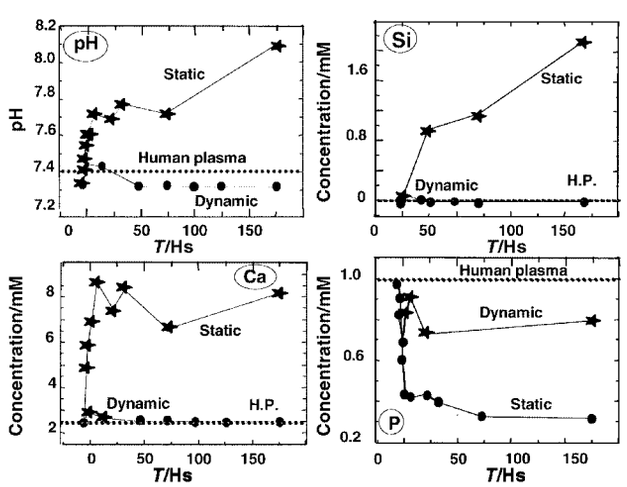



**Fig. 21** Comparison of changes in pH and contents of Ca, Si and P for the same bioactive glass, when the *in vitro* assay was performed under static or dynamic conditions. Data compared with values of human plasma (H. P.).

the concentration of calcium increased in a drastic way during the first 10 hours of soaking, rising from 2.5 to 8.5 mM; afterwards the variations detected were smaller. Simultaneously, the pH increased from the initial value of 7.3 to 7.7 after 10 hours, with a final value of 8.1 after 7 days of assay. However, in dynamic mode, the ionic variations in the solution were negligible, remaining in all cases close to the initial values of SBF.

*Static study.* The variations of the ionic concentration in solution are a consequence of two opposite processes: (a) leaching of ions from the glass to the SBF, increasing the calcium and silicon concentrations, (b) growth of the calcium phosphate layer, which decreases the calcium and phosphorus concentrations in solution. Since SBF does not contain silicon and 80S20C does not contain phosphorus, following the variations of these elements allowed us to assess the evolution of processes (a) and (b). As depicted in Fig. 21, the variation of silicon concentration indicates that its release to solution is somewhat regular. After 7 days, 2% (in weight) of silicon in the glass passed to the solution. The evolution with time of phosphorus in SBF showed a marked decrease during the first 24 hours. After that approximately 30% of the initial phosphate ions remained in solution.

The calcium variation is more complex, since processes (a) and (b) have opposite effects for this ion. The quick initial increase of calcium (Fig. 21) indicates that the ion release to the solution is predominant during the first 10 hours. After that about 21% (in weight) of the calcium in the glass had been released to the solution. Later fluctuations in the calcium concentrations could indicate that, for periods longer than 10 hours, processes (a) and (b) reach a certain equilibrium. However, the variation in the phosphorus concentration led us to state that during the first 10 hours actually more than 21% (in weight) of calcium in the glass was leached to the solution, because a part of it returned to the glass surface as a calcium phosphate.

In addition, with the increase of pH and calcium concentration in solution, the ionic activity product of apatite increased. These phenomena can be considered as general when the *in vitro* behaviour of gel glasses of CaO–$SiO_2$ and CaO–$SiO_2$–$P_2O_5$ systems is studied. Therefore, regarding the ionic concentration, the apatite formation is more favourable under static mode conditions than under *in vivo* situations; this fact questions the validity of the extrapolation of *in vivo* results. Therefore, it is not possible to know if the differences in the apatite formation when comparing *in vitro* studies are due to the properties of the respective glasses or to the ionic variations of the solutions. For this reason, the dynamic protocol seems to be more appropriate.

*Dynamic study.* In this case leaching of glass ions to the SBF (a), calcium phosphate formation (b), and renewal of the solution (c) affected the ionic variations in solution. Indeed, the purpose of solution renewal was to eliminate such variations produced as a consequence of processes (a) and (b). In this way the process will extract the excess of calcium and silicon

ions in the solution, released from the glass, while adding the phosphate ions consumed during formation of the calcium phosphate layer. Indeed, as can be observed in Fig. 21 for the dynamic studies, the ionic concentration and pH of the solution were almost equal to those of human plasma during the whole assay.

It can be summarised that the *in vitro* study of bioglasses allows one to confirm their reactivity with SBF and, as a result, the surface growth of an almost amorphous layer with composition Ca–P–$CO_3^{2-}$, very similar to the mineral component of biological carbonate hydroxyapatite. Therefore, we can consider these bioglasses as bioactive, that is they react chemically with physiological fluids enabling the growth of a phase similar to the mineral component of bone.

Each of the techniques used in these assays, individually, would not allow confirmation of which phase is formed on the surface of these bioactive glasses, due to the nearly amorphous nature of the layer formed. This low crystallinity hinders its precise characterisation by X-ray diffraction; SEM and EDS yield information that, by itself and without further techniques, would be far from conclusive. The chemical analyses reveal the ion exchange that takes place in the soaking process, which again would be incomplete. FTIR allows one to monitor from the very first hours of assay the formation of P–O and C–O bonds. It is the combination of all these techniques, with their combined results, which allows affirmation that the phase formed is a carbonate hydroxyapatite similar in composition and crystallinity to the biological apatite.

If the bioactivity assays are performed *in vitro* without any cell content in the solution, the final product grown on the surface of the bioglass is a carbonate hydroxyapatite; but if the assay is performed *in vivo* the final product will be newly formed bone (Fig. 22).

The bioactive glasses, right after starting to be soaked in body fluids, perform a surface ionic exchange. This consists of a release of calcium ions, forming in a few minutes a silica gel layer. In a second stage the formation of carbonate hydroxyapatite takes place, hardly distinguishable from the biological apatites found in our bones and teeth. If the assay is carried out under *in vivo* conditions the collagen produced by part of the cells will be added to the newly formed surface layer to produce an adherent interface with the glass, where the osteoblasts will begin to deposit bone.[82] This is the fundamental reason why these materials, the bioactive glasses, are being used in periodontal repair, vertebral replacement, bone repair and bone augmentation. However, the bioactive glasses in practical use nowadays (several of them are already commercial products) have been obtained by melting techniques. Bioactive glasses obtained by sol–gel methods are not authorised yet for application in human clinical treatments. The obtaining of such authorisation, in the case of human applications, is a lengthy process. The authorisation protocol is nevertheless underway. This should empower the application of such materials, since it is known that the growth kinetics of carbonate hydroxyapatite is significantly higher than that observed in melted glasses, as well as the bone growth rate found in all preliminary assays performed in animals.

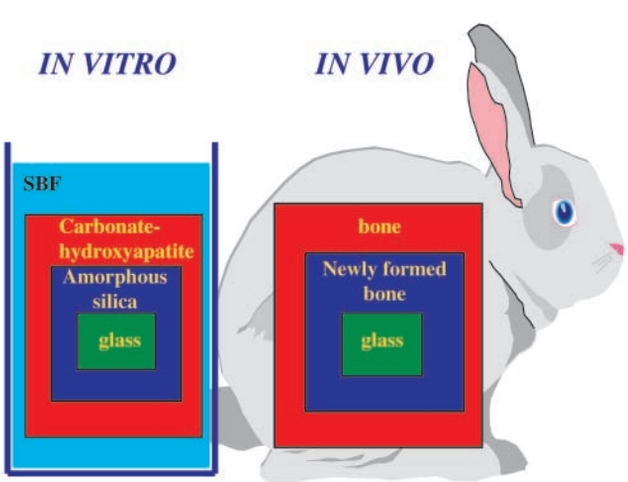



**Fig. 22** Comparative diagram of *in vitro* and *in vivo* assays of a bioactive glass.

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