Synthesis of spherical calcium phosphate particles for dental and orthopedic applications

Marc Bohner,† Solène Tadier,* Noémie van Garderen, Alex de Gasparo, Nicola Döbelin* and Gamal Baroud

Calcium phosphate materials have been used increasingly in the past 40 years as bone graft substitutes in the dental and orthopedic fields. Accordingly, numerous fabrication methods have been proposed and used. However, the controlled production of spherical calcium phosphate particles remains a challenge. Since such particles are essential for the synthesis of pastes and cements delivered into the host bone by minimally-invasive approaches, the aim of the present document is to review their synthesis and applications. For that purpose, production methods were classified according to the used reagents (solutions, slurries, pastes, powders), dispersion media (gas, liquid, solid), dispersion tools (nozzle, propeller, sieve, mold), particle diameters of the end product (from 10 nm to 10 mm), and calcium phosphate phases. Low-temperature calcium phosphates such as monetite, brushite or octacalcium phosphate, as well as high-temperature calcium phosphates, such as hydroxyapatite, β-tricalcium phosphate or tetracalcium phosphate, were considered. More than a dozen production methods and over hundred scientific publications were discussed.

Every year, millions of human beings suffer from a bone loss caused by trauma and illnesses. The standard treatment of care consists in filling the bone defect with a material to support new bone formation. In most cases, autologous bone transplant is used.1,2 Unfortunately, extracting this material requires a second surgery and may lead to complications such as infections or long-lasting pains.3-5 In fact, most studies report a complication rate close to 20%. So, the scientific community has made large efforts over the past 40 years to find suitable bone graft substitutes.6-8 All types of materials have been considered and tested such as polymers,9-10 ceramic-polymer composites,9 metals,11,12 glasses,8 or ceramics.2-7 However, since human bone consists of 65% carbonated apatite, most research efforts have been focused on calcium phosphates (CaPs), in particular hydroxyapatite [HA; Ca₁₀(PO₄)₆(OH)₂], β-tricalcium phosphate [β-TCP; Ca₃(PO₄)₂], and their composites called biphasic calcium phosphates (BCP)2,3 (Table 1). These materials have an excellent biocompatibility, osteoconductivity, and osteotransductivity.14,15 Also, recent reports have demonstrated an osteoinductive potential.16-19 CaP based bone graft substitutes are available in different forms such as granules, porous blocks, cements, “putties” (= non-setting slurries or pastes), sponges/foams, or strips/membranes.2,20 Granules are by far the most frequently used materials due to their relatively low cost,20 broad availability, and good biological properties. Indeed, the inter-granular space is rapidly invaded by newly-formed bone and ceramic resorption can proceed fast and throughout the defect.21 So, the use of non-granular materials remains fairly marginal and is often limited to very specific indications. For example, porous blocks are particularly adequate when the bone defect has a geometrically well-defined shape, such as in open-wedge tibia osteotomy22 or after bone extraction with a trephine system.23 Porous blocks are also extensively used in tissue engineering as scaffolds for cells.24 In the 1990s, the so-called CaP cements (CPCs) raised hopes to eventually replace metals as raw materials for internal fixators.25 Unfortunately, CPCs have several important drawbacks such as their high cost,26 brittleness,26 and slow resorption rate.27 Nevertheless, they can be implanted by minimally invasive techniques,25 and provide mechanical support.27 Also, they have been considered for the treatment of vertebral bone fractures, especially in young patients.28,29 The three other forms of CaP bone substitutes mentioned herein (putties, sponge, membrane) are generally in the form of a polymer matrix filled with CaP particles. Among these three forms, putties have perhaps the best commercial potential, because they often have biological properties as good as those of CaP granules,23 but a much better handling. However, putties have such a broad range of compositions, rheological properties, and biological responses30 that it is difficult to describe their properties succinctly. On one hand, putties may consist of nanoparticles dispersed in an aqueous solution.31-35 After implantation, the paste is seen as a dense solid by cells. Cell invasion is possible but requires material displacement or removal.35 On the other hand, putties may consist of granules held together by a hydrogel, hence allowing rapid bone ingrowth into the space filled with the hydrogel and ceramic resorption throughout the defect.21,36 Even though the first CaP putties were proposed in the 1990s37 and appeared only a decade ago in Western countries,33,34 numerous CaP putties have been launched in recent years.30 This
and controlling the design of CaP particles is of paramount importance to control the handling properties of putties such as cohesion and injectability. Several approaches can be used to optimize both properties. For example, an increase of the viscosity of the liquid phase improves the paste cohesion and reduces the phase separation between liquid and solid, hence resulting in a better injectability. Another strategy is to decrease the mean particle size, as it is known that pastes made of nanoparticles combine a good injectability and a good cohesion. Unfortunately, a reduction of the mean particle size is not always possible. For instance, α-TCP and β-TCP powders which are generally obtained by dehydration of MCPM just above 100°C, can also be obtained by precipitation in organic media.

The first phases can be obtained at or close to room temperature: they are called "Low-temperature CaPs." The last 6 phases can only be obtained at temperatures above 100°C and hence are called "High-temperature CaPs." Thermodynamically, hydroxyapatite (HA) is the most stable phase above a pH value close to 4.5 but only readily precipitate above pH 7.0–7.5. Interestingly, the Ca/P molar ratio of precipitated HA (PHA) tends to vary according to the synthesis conditions, being lower in neutral pH conditions than in basic pH conditions. When the Ca/P molar ratio is equal to 1.50, one refers to "calcium-deficient hydroxyapatite" (CDHA). The typical size of PHA crystals is below 100 nm. Since HA is stable at high temperature, HA can also be formed by solid state reaction. Even though the composition is the same as that of PHA, the crystal size is much bigger. *Could be also classified under "low-temperature CaPs" because MCP can be obtained by dehydration of MCPM just above 100°C. **Can also be obtained by precipitation in organic media. ***Very difficult to synthesize because it is extremely hygroscopic.

The evolution of the bone graft substitute market size is driven by the two-digit yearly increase of the bone graft substitute market size but also by a trend toward simplified pre-operative and/or operative handling properties.

All CPCs and putties have a common feature: they consist of singular solid particles that interact physically and/or chemically during handling. As such, understanding these interactions and controlling the design of CaP particles is of paramount importance to control the handling properties of putties such as cohesion and injectability. Several approaches can be used to optimize both properties. For example, an increase of the viscosity of the liquid phase improves the paste cohesion and reduces the phase separation between liquid and solid, hence resulting in a better injectability. Another strategy is to decrease the mean particle size, as it is known that pastes made of nanoparticles combine a good injectability and a good cohesion. Unfortunately, a reduction of the mean particle size is not always possible. For instance, α-TCP and β-TCP powders which are generally obtained by high-temperature solid-state reactions and milling, do not become smaller during prolonged milling but amorphous. Also, most granular CaP bone graft substitutes have a diameter in the range of 0.5 to 5 mm because they should allow blood vessel in-growth in the intergranular space, hence providing rapid ceramic resorption and bone in-growth.

A third strategy to improve injectability is to use spherical particles. Indeed, Ishikawa et al. showed that the injectability of a CPC paste can be enhanced using spherical tetracalcium phosphate particles. Unfortunately, manufacturing spherical CaP particles is not easy and despite a rapid increase in papers devoted to spherical CaP particles, it is often difficult to know which method to select. Therefore, the aim of the present document is 2-fold: (1) the main emphasis is set on the review of methods used to produce spherical CaP particles, from nanoto milliparticles, and from low-temperature to high-temperature CaP phases; (2) a minor emphasis is set on the review of studies.
in which spherical CaP particles have been used. The next two sections of the present article will address these two aims.

To identify the relevant literature, a search was performed in Scopus (www.scopus.com) with the following search criteria: (spherical OR sphere OR round) AND (calcium phosphate OR apatite OR calcium hydrogen phosphate) AND (granule OR bead OR particle). Over 500 documents were found (March 14, 2013). Additional articles which did not show up in the literature search but which are relevant for the present review were also considered.

Very often, several names have been given to the same or to very similar production methods. For simplicity reasons, similar production methods were grouped under one name (e.g., “drip casting” instead of “droplet extrusion,” or “plasma melting” for “combustion flame spraying” and “flame spheronization”) (Table 2).

Methods to Produce Spherical CaP Particles

More than a dozen different methods have been used to produce spherical CaP particles. Considering this large body of information, it is difficult to provide a short and clear overview. In an attempt to solve this problem, the different production methods were classified according to various criteria: (1) the types of reagents used to produce spherical particles; (2) the media in which the spherical aggregates/particles were dispersed or formed; (3) the dispersion tools; (4) the consolidation reactions that led to solid spherical particles; (5) the typical diameters of the resulting particles; and (6) the CaP phases that could be obtained (Table 2). The next lines are devoted to these various aspects.

Reagents. In principle, there are four types of reagents used in the production of spherical CaP particles: solutions, slurries (= suspensions), pastes, and powders. Most methods are either based on solutions, slurries, and pastes because it is easier to control the dispersion of a fluid than that of a solid. In this document, it is assumed that a slurry flows under its own weight contrary to a paste.

Solution-based methods are the most adequate to produce nanoparticles (<100 nm). For example, several authors have reported the production of spherical CaP particles with a diameter close to 15–20 nm, either by precipitation, spray-drying, and flame-synthesis (= flame pyrolysis) (Table 2). The solutions may be either aqueous (precipitation, spray-drying), non-aqueous (precipitation, flame-synthesis) or a mixture thereof (precipitation-emulsification).

Slurry-based methods have many similarities with solution-based methods except that the liquid already contains dispersed particles. Therefore, the spherical particles produced from slurries generally consist of agglomerates of the primary particles dispersed in the slurry, and their diameter is often a few orders of magnitude bigger than the diameter of the primary particles (Table 2). For example, 1 mm particles can be obtained by drip-casting (= droplet extrusion), emulsification, and hydro-casting, and by the lost-wax method (Fig. 2). The main exception to this rule is the method called suspension plasma spraying (= atomization), in which a CaP suspension is injected into a high-energy plasma torch and then sprayed at high energy through a nozzle to form particles in the range of 10 nm to 100 μm.

In all slurry-based methods, the consolidation stage is very important because slurries lose their shape under gravity conditions for more information, see the section devoted to the consolidation stage. This is the main difference compared with paste-based methods. Indeed the latter methods rely on the ability of the pastes to behave like solids. For example, in the extrusion-spheronization process, pastes are extruded through a die to form rods. These rods are then spun on a horizontal plate covered by small truncated pyramids (roughly 1–2 mm in height) (Fig. 3). The mechanical interactions between the rods, and these truncated pyramids lead to the rupture of the rods into small segments, which are then rounded by the rotational movements and the interactions with the truncated pyramids. Since all paste-based methods (i.e., spray granulation, extrusion-spheronization, sieve-shaking) involve fairly viscous pastes, the particles obtained by these methods are typically in the millimeter range.

One difficulty in the use of pastes is the adjustment of their rheological properties. Generally, the liquid amount has to lie just below (spray granulation, sieve shaking) or just above (extrusion-spheronization) the plastic limit of the powder (the plastic limit is defined as the minimum amount of water that has to be added to a powder to get a paste). However, the plastic limit may vary and small deviations may provoke drastic changes of the paste viscosity, in particular for powders with a fairly large mean particle size (>1–10 μm). Also, the paste has to be viscous but not sticky. This is a particularly difficult task for extrusion spheronization, and explains why only very few binders can be used.

Among the different reagents, powders are solely used in the process called “plasma melting” (also called “combustion flame spraying,” and “flame spheronization”) during which particles are injected into gas plasma. The high temperature melts the particles into spherical droplets. Subsequently, these droplets are consolidated by freezing. As a result, the particle size distribution of the processed particles depends mainly on the initial particle size distribution and little can be done to control it during the process.

All the methods listed in Table 2 are top-down approaches, i.e., a large volume is divided into smaller volumes. In recent years, quite a few bottom-up approaches have been proposed, the so-called solid free form fabrication (SFFF) methods, in which small solid subunits are piled up in a controlled manner to form complex 3D architectures. One of these methods, called 3D printing, relies on the spatially-controlled reaction of a liquid with a solid. For example, Gbureck et al. printed CaP porous scaffolds by jetting phosphoric acid onto an α-TCP powder bed. Even though SFFF methods are primarily used for the synthesis of complex objects, they can also be applied to the production of small particles (Fig. 2). However, resolution is relatively limited, so the production of CaP spheres smaller than 1 mm is possible but difficult.
Table 2. Classification of the methods used to produce spherical CaP particles according to the types of reagents, the dispersion media, the dispersion tools, the consolidation methods, the resulting diameters, and the final composition

| Reagents | Dispersion media | Dispersion tool | Consolidation | Method name | Diameter | Composition |
|----------|------------------|----------------|--------------|-------------|----------|-------------|
| Solution | No dispersion    | -              | Precipitation| Precipitation\(^{67, 90, 102, 103, 110, 115, 118, 125, 131, 156-161}\) | 0.01–1,000 μm | DCPD\(^{90}\) |
|          |                  |                |              | OCP\(^{102, 128, 131, 156, 159}\) |          |             |
|          |                  |                |              | ACP\(^{97, 103, 112, 114, 125}\) |          |             |
|          |                  |                |              | β-TCP\(^{96}\) |          |             |
|          |                  |                |              | HA\(^{47, 48, 102, 110-115, 125, 127, 156-160, 161}\) |          |             |
| Gas (Aerosol) | Nozzle (high energy) | Pyrolysing and drying | Flame-synthesis\(^{57-60, 162-165}\) ( = spray pyrolysis) | 0.01–6 μm | MCPM\(^{98}\) |
|          |                  |                |              | DCP\(^{99}\) |          |             |
|          |                  |                |              | ACP\(^{102, 162}\) |          |             |
|          |                  |                |              | β-TCP\(^{97}\) |          |             |
|          |                  |                |              | HA\(^{57-60, 163-165}\) |          |             |
|          | Nozzle (high energy) | Drying | Spray-drying\(^{52}\) | 0.1–5 μm | HA52 |
|          |                  |                |              | Electrospraying\(^{106}\) | 1–7 μm | β-TCP\(^{106}\) |
| Liquid (Emulsion) | Propeller | Precipitation | Precipitation-emulsification\(^{56, 51, 61, 148}\) | 0.02–20 μm | DCPD\(^{92}\) |
| Plasma | Nozzle (high energy) | Freeze | Suspension Plasma-spraying ( = atomization)\(^{90-94}\) | 0.01–100 μm | HA\(^{96-98}\) |
|          | Gas (Aerosol) | Nozzle (high energy) | Drying | Spray-drying\(^{75, 96, 102, 127, 132, 147, 161-171}\) | 0.4–240 μm | DCP\(^{107}\) |
|          |                  |                |              | β-TCP\(^{105, 171}\) |          |             |
|          |                  |                |              | HA\(^{75, 96, 107, 117, 126, 132, 147, 168-171}\) |          |             |
| Slurry | Gas + liquid | Nozzle (high energy) | Freezing | Freeze granulation\(^{104}\) | 0.4–240 μm | HA\(^{104}\) |
|          | Nozzle (low energy) | Gelling\(^{62, 65, 68, 172}\) | Drip casting\(^{62, 71}\) = Droplet extrusion | 100–400 μm | BCP\(^{70, 71}\) |
|          | Gelling\(^{51, 76, 173}\) | Drying64 | HA\(^{52, 64-68, 172}\) |          |             |
|          | Propeller | Precipitation\(^{72, 75, 79, 82, 106, 174}\) | Emulsification\(^{72, 82, 106, 108, 174–176}\) | 50–6,000 μm | DCPD\(^{72, 106}\) |
|          | Liquid | Gelling\(^{72, 74, 79, 77, 108, 174}\) | HA\(^{72, 74, 75, 77, 81, 106, 174–176}\) |          |             |

The column entitled “method name” contains one or several names used to call the production method. The production methods are either based on solutions, slurries, pastes, or powders. Here, a difference is made between slurries (low-viscosity, free-flowing) and pastes (high viscosity). Formation of spherical particles occurs either in a plasma, a gas, a liquid or a solid using nozzles, propellers, sieves, or templates. A “high energy” dispersion is used to describe a highly turbulent dispersion regime, in contrast with a “low energy” dispersion regime occurring in laminar flow conditions. The consolidation steps may involve precipitation, drying, pyrolysis, gelling, or freezing. The diameter may range between 0.01 μm and a few millimeters. Finally, all types of CaP phases can be produced, but not all methods can be used to produce one particular CaP phase. This table is only considering published methods used to produce CaP particles. Many other methods have been proposed, in particular with pelletizers\(^{154, 155}\) and bottom-up approaches\(^{106}\) such as 3DP\(^{98, 100}\).


Before ending this section, it is worth mentioning that spherical particles can also be obtained by the so-called double dispersion method in which a calcium-rich solution is separated from a phosphate-rich solution by a permeable membrane such as a gel. It can be considered as a type of precipitation reaction.

**Dispersion media.** With the exception of precipitation reactions, all production methods rely on the dispersion of the reagent (solution, slurry, paste, or solid) into another phase: a gas to form an aerosol, a liquid to form an emulsion, or a solid acting as a mold (lost-wax method). Whereas slurries have been used in all three dispersion approaches, solid reagents have only been dispersed in a gas (plasma melting). Due to their relatively low concentrations, solutions have not been used in the lost-wax method because it would only coat the walls of the template (or mold) instead of filling the template.

Interestingly, some methods combine different approaches. For example, Liu et al. used a drip casting approach to deposit a syringe a slurry into hemispherical molds (= “lost-wax” method). Also, some production methods rely on two dispersion media: a gas and a liquid (e.g., for freeze-granulation and drip casting), or two immiscible liquids such as in hydro-casting.

**Dispersion tools.** Generally, three dispersion tools are used for the production of spherical CaP particles (Fig. 3): (1) nozzles to disperse solutions and slurries into a gas, (2) sieves to granulate pastes, and (3) propellers to produce emulsions and to granulate wet powders. Depending on the dispersion energy, strong changes of the final particle diameter can be achieved. This is particularly true for nozzle-based dispersion methods. In laminar conditions (e.g., in drip casting), the mean diameter of the produced droplets is equal to 2–5 times the nozzle diameter (Fig. 3). Also, there is a good control of the particle diameter and the particle size distribution is narrow. For example, Ribeiro et al. reported the synthesis of HA particles with a mean diameter of 500 μm and a size dispersion lower than 10% (the size dispersion is defined by the ratio between standard deviation and mean of the particle size distribution). Even better results were obtained by Teraoka and Kato by hydro-casting, a method similar to drop-casting but with two small distinctions: the slurry is injected into a liquid instead of a gas and the injected amount is controlled with a micropipette. Specifically, these authors produced 1.3 mm α-TCP particles with a dispersity of 1.4% and a sphericity of 1.01 (ratio between long and short axis). On the negative side, it is difficult to obtain diameters below 100 μm due to nozzle plugging. Interestingly, the same problem restricts the performance of sieve-based production methods such as extrusion-spheronization or sieve-shaking (Table 2). At high dispersion energy (turbulent flow), the droplet diameters can be much smaller than the nozzle diameters. So, it is possible to obtain much smaller particles than by low-energy approaches. However, the particle size distribution is broad. For example, Andrianjatovo et al. spray-dried β-TCP particles with a diameter in the range of 0.4 to 237 μm.

**Table 2.** Classification of the methods used to produce spherical CaP particles according to the types of reagents, the dispersion media, the dispersion tools, the consolidation methods, the resulting diameters, and the final composition (continued)

| Reagents | Dispersion media | Dispersion tool | Consolidation | Method name | Diameter | Composition |
|----------|------------------|----------------|---------------|-------------|----------|-------------|
| Slurry   | Liquid + liquid  | Nozzle (low energy) | Gelling | Hydro-casting | > 1,000 μm | α-TCP83 |
|          | Solid            | Template or mold | Drying | Lost wax84,85,121 | 300–3,000 μm | BCP21 |
| Paste    | Gas              | Propeller | Drying | Spray-granulation95,90 (high-shear mixing) | 100–8,000 μm | DCPD93 |
|          | Solid            | Sieve      | Drying | Extrusion-spheronization89,91 | 500–2000 μm | DCPD93 |
| Powder   | Plasma           | Nozzle | Freezing | Plasma melting45,46,94-97 | S-125 μm | TetCP45,46 |

The column entitled “method name” contains one or several names used to call the production method. The production methods are either based on solutions, slurries, pastes, or powders. Here, a difference is made between slurries (low-viscosity, free-flowing) and pastes (high viscosity). Formation of spherical particles occurs either in a plasma, a gas, a liquid or a solid using nozzles, propellers, sieves, or templates. A “high energy” dispersion is used to describe a highly turbulent dispersion regime, in contrast with a “low energy” dispersion regime occurring in laminar flow conditions. The consolidation steps may involve precipitation, drying, pyrolysis, gelling, or freezing. The diameter may range between 0.01 μm and a few millimeters. Finally, all types of CaP phases can be produced, but not all methods can be used to produce one particular CaP phase. This table is only considering published methods used to produce CaP particles. Many other methods have been proposed, in particular with pelletizers and bottom-up approaches such as 3DP.
achievement of a given droplet size. Other factors such as the stirring rate, the emulsifier type and concentration, or the viscosity of the two immiscible liquids are much more important.73,106

In the “lost-wax” method, spherical particles can be produced with a mold or a template (the mold or template has to be removed after CaP particle synthesis). In the latter case, the particles are either hollow (after template removal) or biphasic. To the best of our knowledge, there is currently no commercial application requiring such features.

Consolidation Methods

All production methods of spherical CaP particles involve fluids. This is necessary to provide the spherical shape. Immediately after their formation, the particles are still soft and their shape and size are still subject to change. The transition from a soft to a hard state (= consolidation) is critical in order to obtain non-agglomerated, well defined spheres. Therefore, consolidation methods such as drying, freezing, gelling, and crystallization are of paramount importance (Fig. 4).

Drying is by far the most popular approach due to its simplicity. However, there is a size reduction associated with it which may cause density gradients and lead to heterogeneities (e.g., hollow particles during spray-drying107). This problem does not occur by freezing the particles in liquid nitrogen, but frozen particles have to be freeze-dried, which is a rather lengthy and expensive process. Also, the impact of the droplets into liquid nitrogen may impair the particle sphericity. Finally, the absence of shrinkage during freeze-drying is detrimental for the mechanical properties of the granules.

When a liquid is dispersed into another liquid without temperature change, it is not possible to rely on drying or freezing to consolidate the particles. In that case, two approaches can be used: (1) gelling and (2) crystallization. For example, Tuyen et
be used. Specifically, several methods are particularly adapted to obtain narrow size distribution: (1) precipitation methods characterized by burst nucleation and diffusion-controlled growth,\(^3\), (2) drip casting,\(^6\)-\(^7\), (3) hydro-casting,\(^8\), and (4) 3D printing (Fig. 2). For example, Mateus et al.\(^6\) who used drip casting to produce HA particles reported the synthesis of HA particles with a diameter in the range of 0.5 to 0.9 mm and size dispersion lower than 10%. Nevertheless, the narrowest size distributions are obtained by machining approaches, such as hydro-casting and 3D printing, because with these techniques, the size and sometimes also the shape of each particle is controlled individually. However, these methods are currently limited to fairly large particles, typically in the millimeter range. For example, Teraoka and Kato\(^8\)

Particle diameters. Spherical particles can be obtained over a very broad size range, spanning from 10 nm to a few millimeters. Generally, the smallest particles are obtained by solution-based methods (e.g., precipitation, flame synthesis) whereas the biggest particles are produced by paste-based methods (e.g., extrusion-spheronization, spray granulation). Even though all particle sizes can be obtained using several methods, particles in the range of 100 to 300 \(\mu\)m are difficult to produce with high yield and good shape control. Indeed, a diameter of 100 \(\mu\)m is at the upper range of what spray-drying, freeze granulation or plasma melting can successfully achieve whereas 300 \(\mu\)m is at the bottom range of what is feasible with drip casting, extrusion spheronization, 3D printing or the lost wax method (Table 2).

Not all methods can be used to produce spherical CaP particles with a narrow size distribution. Generally, high-energy dispersion tools lead to broad size distributions. For example, Jiao et al.\(^1\) reported a diameter in the range of 0.1 to 60 \(\mu\)m for their spray-dried hydroxyapatite particles. Similarly, Andrianjatovo et al.\(^1\) reported diameters between 0.4 and 237 \(\mu\)m for \(\beta\)-TCP particles. So, low-energy dispersion methods or SFFF methods must
used hydro-casting to produce 1.3 mm α-TCP particles with a
dispersity of 1.4%, and a sphericity of 1.01 (ratio between long
and short axis).

Even though certain production methods provide particle
populations with a narrow size distribution, such populations are
generally classified, for example by sieving.90 This is not only a
way to remove too small or too large particles, but also to control
their sizes.

In this section, the diameter of the particles formed by various
production processes was discussed. However, there is always a
risk of agglomeration that could potentially ruin the efforts to
produce a narrow particle size distribution. In general, small par-
cicles (< 1–10 μm) are much more prone to agglomeration due to
van der Waals forces than larger particles. Also, drying processes,
particularly in the presence of water, are very critical because they
can promote compaction and agglomeration of previously loose
particles.

**Composition.** Not all production methods can be used to pro-
duce one particular CaP phase (Table 2). As a result, the choice of
a particular production method has to be related to the phase that
has to be produced. Generally, high-temperature processes, e.g.,
plasma melting and flame-synthesis, cannot be used to produce
hydrated phases such as DCPD and OCP. Similarly, low-tem-
perature processes, such as precipitation, are not adequate for the
production of α-TCP and TetCP which are only thermodynam-
ically stable above 1100–1200°C. However, there are exceptions.
For example, Mohn et al.91 showed recently that MCPM, DCP
and DCPD were present in flame-synthesized CaP nanoparticles.
The authors assumed that hydration occurred during cooling.
Also Tao et al.35,56 synthesized nanosized β-TCP octahedral and
hexagonal crystals by precipitation in a non-aqueous medium.
β-TCP is generally considered to be a high-temperature phase that
can only be obtained by thermal treatments above 700–800°C.
Another approach to obtain low-temperature CaP phases from
high-temperature CaP phases is to apply post-treatments. Indeed,
Gonda et al.77 demonstrated that α-TCP beads placed into an
autoclave at 160°C converted to CDHA. Similarly, Nomura et
al.106 converted calcium sulfate dihydrate particles into HA parti-
cles. Finally, DCP particles can be obtained by incubating α-TCP
particles in phosphoric acid.109

Obviously, high-temperature phases can be obtained from
low-temperature phases by a simple thermal treatment. In fact,
this strategy is widely used because the application of a thermal
treatment on e.g., spray-dried or drip-casted particles cannot only
remove organic additives used during the manufacturing process,
but also consolidate the particles by sintering. Nevertheless, post-
treatments may lead to aggregation or even deterioration of the
particles. Thus, the use of post-treatments has to be considered
carefully.

As hinted in the previous paragraph, many production meth-
ods listed in Table 2 require the use of additives. The additives
can have different functions, such as growth inhibitors for pre-
cipitations (e.g., proteins57), gelling agents for drip casting (e.g.,
alginites62,65-69), dispersants for the lost wax method, emulsions,
and freeze casting (e.g., ammonium polycrylates73,84,104), bind-
ers for spray-granulation and extrusion spheronization (e.g.,
microcrystalline cellulose49,50), and emulsifiers for emulsions (e.g.,
polyethoxylated castor oil79). In some cases, the use of these addi-
tives is essential (e.g., microcrystalline cellulose for extrusion-
spheronization90,91), but in most cases, additives are only used to
improve the properties of the final product. Since additives
should be removed prior to the clinical use of the CaP particles,
the advantages related to their use should be balanced with the
troubles generated by their removal. Normally, organic remnants
are pyrolyzed.72,84,91 The latter strategy is obviously not applicable
for hydrated CaP phases such as DCPD and OCP, because these
phases decompose well below 500°C. Another possibility is to
dissolve them in an appropriate solvent.50,79 However, it is often
difficult to get rid of all additives, in particular when dealing with
HA. Indeed, precipitated HA crystals are generally very small and
hence present very large specific surface areas (typically above 50
m²/g108-115). Moreover, HA has excellent adsorption properties,
which explains its use in chromatography.116-118

So far, it has been assumed that spherical particles have a
homogeneous composition. However, CaP particles obtained at
high-temperature are prone to heterogeneities.96 For example,
Carayon et al.119 postulated that plasma-sprayed particles are
made of three layers: (1) an apatite core, (2) an intermediate layer
of calcium oxide, TetCP and tricalcium phosphate, and (3) an
outer layer consisting of amorphous calcium phosphate. CaP par-
ticles obtained at low temperatures can also present local hetero-
genieties: Jäger et al.120 showed that precipitated HA nanocrystals
consisted of a crystalline core with a calcium to phosphate ratio
of 1.67 and an amorphous layer with a calcium to phosphate ratio
of 1.00.

**Other aspects.** Beside the aspects described in Table 2, many
other aspects might be relevant for a particular clinical indication,
such as the mechanical stability or the specific surface area of the
spherical particles. A few of these aspects are discussed in the next
cfew lines.

Since this review focused on spherical CaP particles for
clinical use, it is relevant to look at their mechanical stability.
Unfortunately, the determination of the mechanical properties of
spherical particles, particularly of such small diameters, is very
difficult and hence hardly performed.90,121 The few attempts of
mechanical characterization found in the literature hence still
leave room for speculation. It is likely that high-temperature syn-
thesis methods, such as flame-synthesis and plasma melting, pro-
vide the mechanically most stable CaP spherical particles because
sintering and/or crystallization occurs. Contrarily, consolidla-
bition by freeze-drying is expected to produce the most unstable
particles.

If the mechanical stability of the spherical particles is too
low, three main approaches can be used to reinforce them. First,
a binder can be added into the raw materials. Generally, a few
weight percents of a polymer are able to give enough stability for
handling purposes.84 Second, the particles can be sintered. For
example, Paul and Sharma72 sintered chitosan-bonded HA par-
ticles at 1100°C for 1 h to obtain organic-free phase pure HA
particles. Depending on the conditions of sintering, linear shrink-
age can easily reach 20–25% and the particle composition may
change. The third consolidation method involves the hydraulic
or hydrothermal conversion of a CaP phase into another phase. For example, Tas82 converted α-TCP spherical agglomerates into apatite particles by incubation in distilled water for 48 h at room temperature. Similarly Gonda et al.77 autoclaved α-TCP spherical particles for 20 h at 160°C to convert α-TCP grains into interlocked CDHA rods. Very often, several approaches are combined: Takahashi et al.122 produced α-TCP spherical beads with an emulsion route. They used gelatin as a binder, removed it by sintering at 1200°C, and then transformed hydrothermally the α-TCP phase into HA. Here, it is also worth mentioning the work of Day et al.123,124 who first produced spherical glass microspheres and then converted them into hollow apatite spheres by incubation in a phosphate solution.

A large number of studies have been devoted to the synthesis of spherical particles for drug delivery.67,79,74,80,104,125-127 As a result, the internal structure of the particles, such as the porosity, the pore size distribution, or the size distribution of the pore interconnections, is of particular relevance. Various authors have attempted to control it: Descamps et al.84 showed that macro-porous CaP spheres could be produced with a controlled fenestration size between the macropores (Fig. 5). Unfortunately, this approach can only work with fairly large spheres (several millimeters in diameter) and CaP phases stable at high temperature (e.g., β-TCP, HA). Similar granule sizes and macropore dimensions were obtained by Lee and Park86 using double emulsions and by Yang et al.78 using drip casting. At a much lower scale, ≈100 nm hollow apatite particles can be obtained by precipitation either without112-114,125 or with templates.118,128 Finally, hollow particles can also be produced in an intermediate size range (100–1000 μm) using emulsions,81,82 and precipitations.129-131

Drug loading is often achieved during67,80,125,132 or after particle production74,79,80,104,127 depending on the drug and the production method. When drug loading is performed during particle production, it is more difficult to control the production and purity of the particles because the system contains more components. When drug loading is performed after particle production, drug absorption into the particle core might be difficult, in particular if the particles are designed for prolonged drug release (small pores). Drug release is generally controlled by dissolution, diffusion, or surface interactions. In the latter two cases, HA is the most interesting CaP phase because it has often very high specific surface areas, typically above 50 m²/g.110-115 However, this implies that the spherical particles must be produced at or close to room temperature, e.g., by precipitation.110-115 Indeed, high temperature processes, e.g., plasma melting or sintering, would strongly reduce the specific surface area of the HA.

As described in the previous paragraphs, the pore size distribution of spherical particles is of prime importance for drug delivery. For orthopedic and dental applications, the focus is set on the space between the particles. Indeed, blood vessels and cells should be able to invade the inter-particular network to promote ceramic resorption and bone formation throughout the particle-filled defect. So, particles should be big enough to promote an easy blood vessel/bone ingrowth but not too large to keep an acceptable resorption time.133 Zhang et al.80 tried to estimate the size of the intergranular space based on the size of the spherical particles. They came to the conclusion that values close to 1 to 2 mm are ideal for orthopedic and dental applications.

**Use of Spherical CaP Particles**

Besides orthopedics and dentistry (Table 3), spherical CaP particles are used in very diverse fields of application such as food industry, pharmaceutics, or agriculture (Table 4). For the latter applications, very large volumes are produced and sold. So, in terms of the production volume, the use of CaPs in the medical industry can be considered as a niche application. However, the sale prices are much higher than in other applications and the market is rapidly growing.20 Also, the types of CaPs used in orthopedics and dentistry are much more diverse than those used in other fields. Indeed, not only low-temperature but also high temperature phases such as β-TCP, α-TCP or TetCP are used, either as raw materials for hydraulic cements or as bone substitutes.134

So far, the number of commercial products based on spherical CaP particles and used in orthopedics and dentistry is still limited (Table 5). However, this number is increasing. Moreover, the rapid increase in scientific publications in this field of research (Fig. 1) suggests that new commercial products will be launched soon. From the list of medical products given in Table 5, only three products are applied in an injectable form. For example, “Radiance,” a product used in aesthetic surgery, contains 30% volume HA in a CMC gel.135,136 Similarly, the orthopedic cement called “chronOS Inject” contains roughly 20% volume β-TCP beads in its paste. Finally, no information about the ceramic volume fraction could be found for another injectable bone substitute called “MBCP Gel.”

The type of application in which CaPs are used depends on the CaPs properties, mainly solubility, acidity, and nanostructure.134 MCP and MCPPM are the most soluble and most acidic CaPs. So, MCP/MCPP are used as fertilizer in agriculture.137,138 as food additives (e.g., in combination with sodium bicarbonate to make a leavening agent139), and as raw material for hydraulic cements in orthopedics.140,141 DCP and DCPD are neutral and have an intermediate solubility (the human serum
a product is often related to its ease of handling. In orthopedics and dentistry, spherical particles are generally mixed with a liquid and then injected. It is therefore crucial to understand the rheological properties of such mixtures, and in particular prevent phase separation. The injectability of CaP-based pastes has been addressed in many articles. However, most studies have been devoted to CPCs which are characterized by a mean particle size in the low micrometer range (typically below 10 μm), whereas most CaP particles used for bone graft substitution are much bigger, typically above 100 μm (“milliparticles”). Since many putties and some CPCs consists of CaP milliparticles embedded in a paste, it is essential to better understand the role of milliparticles on the injectability of such pastes. Questions that can be raised are: (1) Beyond what volume fraction of milliparticles is injectability impaired? (2) What is the importance of milliparticle size on injectability? (3) Are the lessons drawn from CPCs valid for pastes made of milliparticles? Tadier et al. tried to address some of these questions in a recent study using a model paste consisting of β-TCP microparticles (mean size ≈10 μm), water and glass milliparticles (mean size in the range of 100 to 400 μm). The results confirmed most previous findings made

Table 3. Applications of spherical calcium phosphate particles in orthopedic and dental surgery

| Application                                                                 | Size                      | Phase         |
|------------------------------------------------------------------------------|---------------------------|---------------|
| Raw material for calcium phosphate cements                                   | 0.01–500 μm               | DCP<sup>99</sup>  |
| Raw material for 3D printing                                                 | 5–50 μm                   | HA            |
| Raw material for calcium phosphate putties                                    | > 50 μm                   | β-TCP         |
| Raw material for composite materials                                          | < 0.2 μm                  | HA            |
| Raw material for isostatic pressing                                          | 0.1–5.8 μm                | HA            |
| Powder feedstock for plasma spray                                            | 0.5–200 μm                | HA            |
| Bone graft substitute                                                        | > 50 μm                   | DCP<sup>106</sup>  |
| Drug carrier for bone applications (e.g., infections, non-unions, osteoporosis) | > 50 μm                   | BCP           |
| Model particles to induce metalloproteinase and mitogenesis (osteoarthritis) | 17–106 μm                 | Fluoroapatite |
| Cell transfection (gene delivery)                                            | 17–106 μm                 |               |
| Cell carrier (Tissue engineering)                                            | 0.1–850 μm                |               |

is in equilibrium with DCP<sup>42</sup>). Accordingly, DCP and DCPD are used as excipients for tablets, as food additives (for example as phosphorus or calcium source<sup>43</sup>), and as bone substitutes.<sup>44,109,144</sup> CaP materials with slightly larger Ca/P molar ratios (1.33 to 1.67) have been the most extensively used CaPs due to two features: (1) first, their composition and properties are very close to those of bone mineral; (2) second, these materials easily precipitate in the form of nanocrystals. As a result, their specific surface area is very large (typically above 50 m<sup>2</sup>/g), which is of great interest for chromatography,<sup>52,110,113,116,117</sup> drug delivery,<sup>79,104,126,127</sup> or heavy ion capture. Also, nanocrystals have strong van der Waals interactions, hence leading to the formation of very cohesive and viscous aqueous pastes.<sup>10</sup> This can be used to thicken soups and yoghurts. TetCP is the most basic and also one of the most soluble CaP (behind MCP and MPCM). Therefore, it is a very good raw material for hydraulic CaP cements.<sup>149</sup> To the best of our knowledge, it is not used in other applications than orthopedics and dentistry.

So far, the manuscript has been focused on the synthesis and use of spherical CaP particles. The next paragraphs will address the delivery of the particles. Indeed, the commercial success of

Table 3. Applications of spherical calcium phosphate particles in orthopedic and dental surgery

| Application                                                                 | Size                      | Phase         |
|------------------------------------------------------------------------------|---------------------------|---------------|
| Raw material for calcium phosphate cements                                   | 0.01–500 μm               | DCP<sup>99</sup>  |
| Raw material for 3D printing                                                 | 5–50 μm                   | HA            |
| Raw material for calcium phosphate putties                                    | > 50 μm                   | β-TCP         |
| Raw material for composite materials                                          | < 0.2 μm                  | HA            |
| Raw material for isostatic pressing                                          | 0.1–5.8 μm                | HA            |
| Powder feedstock for plasma spray                                            | 0.5–200 μm                | HA            |
| Bone graft substitute                                                        | > 50 μm                   | DCP<sup>106</sup>  |
| Drug carrier for bone applications (e.g., infections, non-unions, osteoporosis) | > 50 μm                   | BCP           |
| Model particles to induce metalloproteinase and mitogenesis (osteoarthritis) | 17–106 μm                 | Fluoroapatite |
| Cell transfection (gene delivery)                                            | 17–106 μm                 |               |
| Cell carrier (Tissue engineering)                                            | 0.1–850 μm                |               |

is in equilibrium with DCP<sup>42</sup>). Accordingly, DCP and DCPD are used as excipients for tablets, as food additives (for example as phosphorus or calcium source<sup>43</sup>), and as bone substitutes.<sup>44,109,144</sup> CaP materials with slightly larger Ca/P molar ratios (1.33 to 1.67) have been the most extensively used CaPs due to two features: (1) first, their composition and properties are very close to those of bone mineral; (2) second, these materials easily precipitate in the form of nanocrystals. As a result, their specific surface area is very large (typically above 50 m<sup>2</sup>/g), which is of great interest for chromatography,<sup>52,110,113,116,117</sup> drug delivery,<sup>79,104,126,127</sup> or heavy ion capture. Also, nanocrystals have strong van der Waals interactions, hence leading to the formation of very cohesive and viscous aqueous pastes.<sup>10</sup> This can be used to thicken soups and yoghurts. TetCP is the most basic and also one of the most soluble CaP (behind MCP and MPCM). Therefore, it is a very good raw material for hydraulic CaP cements.<sup>149</sup> To the best of our knowledge, it is not used in other applications than orthopedics and dentistry.

So far, the manuscript has been focused on the synthesis and use of spherical CaP particles. The next paragraphs will address the delivery of the particles. Indeed, the commercial success of
solid), the dispersion tools (nozzles, propellers, sieves, molds), and the consolidation methods (drying, precipitation, gelling, freezing). As a result of this diversity, a broad range of properties can be obtained, for example in terms of particle diameters (10 nm to 10 millimeters), particle size distributions (very narrow to broad), porosities (low to high), or compositions (all known CaP phases).

This review also revealed that there has been a very rapid increase in the number of research articles since 2003. This has resulted in new approaches to better control the production of spherical CaP particles, for example with hierarchical structures or with very narrow particle size distributions.

Table 4. Some selected applications of spherical calcium phosphate particles in other fields than orthopedic and dental applications

| Application                              | Typical size | Phase     |
|------------------------------------------|--------------|-----------|
| Aesthetic surgery (e.g., skin filling)   | 25–40 µm     | BCP@50    |
| Nerve Regeneration                       | 30–45 µm     | HA@105,136|
| Food (suspension stabilizer, mineral enrichment, baking agent) |              |           |
| Pharmaceutics (e.g., pellets/tablets)   | 1–4 µm @18   | DCP@33    |
| Chromatography                           | 0.2–16 µm    | HA@14     |
| Immuno-adsorbent                         | 200–400 µm   | HA@      |
| Drug                                      | 14 µm        | OCP@      |
| Environment–heavy ion capture, ion exchanger | 10 µm      | HA@105,148|
| Catalyst carrier                         | 1 µm         | HA@      |
| Agriculture/fertilizer                   |              | MCP@132,138|

Table 5. Non exhaustive list of commercial products containing spherical calcium phosphate particles

| Product name and company                  | Description                     | Application       |
|------------------------------------------|----------------------------------|-------------------|
| Calcibon Granules (Biomet)               | Spherical CDHA particles (company website and 92) | Orthopedics       |
| Calc-i-oss (Degradable Solutions)        | Spherical β-TCP particles (company website) | Dentistry         |
| Cerasorb (Curasan)                       | Spherical β-TCP particles @18   | Dentistry         |
| chronOS Inject (DePuySpine)              | Brushite calcium phosphate cement loaded with spherical β-TCP particles @177,178 | Orthopedics       |
| Hydros (Biomatlante)                     | Rounded BCP particles in water @104 | Orthopedics       |
| MBCP Gel (Biomatlante)                   | Rounded BCP particles in an HPMC gel | Orthopedics       |
| Radiance (BioForm)                       | HA particles dispersed in a CMC gel @105,136 | Aesthetic Surgery |

Conclusion

The aim of this article was to review the methods used to produce spherical CaP particles and to look at the use of such particles in the orthopedic and dental fields. Over a dozen of production methods were identified and described. The various methods were classified according to the starting materials (solutions, slurries, pastes, and solids), the dispersion phases (gas, solution, with microparticles. For example, injectability dropped beyond 35–40% milliparticle volume fraction. Also, the latter volume fraction threshold decreased with an increase in particle size. Furthermore, the β-TCP and water phases separated at a constant rate during injection. Moreover, phase separation was reduced with the replacement of water with a viscous aqueous gel. However, a surprising result was observed: the glass microparticles were extruded faster than the β-TCP microparticles. In other words, not all lessons learned from CPC pastes can be translated to pastes containing milliparticles.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors would like to thank L. Galea and J. Thüring for Figure 2A and 2I, and the authors of Figure 5A–E for the permission to use their photos.
66. Oliveira SM, Barrias CC, Ribeiro CC, Almeida IF, Mateus AYP, Barrias CC, Ribeiro C, Ferraz MP, Liu DM. Fabrication and characterization of porous Fabbri M, Celotti GC, Ravaglioli A. Granulates based Barrias CC, Ribeiro CC, Barbosa MA. Adhesion and Hirai T, Hodono M, Komasawa I. Preparation of Barrias CC, Ribeiro CC, Barbosa MA. Preparation and Liu DM, Ogura K, Shibata Y, Shimizu H, Yumura H, et al. Preparation of hydroxyapatite for bone substitutes. J Eur Ceram Soc 2009; 29:369-75; http://dx.doi.org/10.1016/j.jeurceramsoc.2008.06.008. Meurice E, Leriche A, Hornez JC, Bouchat F, Rgiuri E, Boilet L, et al. Functionalisation of porous hydroxyapatite for bone substitutes. J Eur Ceram Soc 2012; 32:6207-8; http://dx.doi.org/10.1016/j.jeurceramsoc.2012.01.014. Bouyer E, Girzhofer F, Boulou MI. Suspension plasma spraying for hydroxyapatite powder preparation by RF plasma. IEEE Trans Plasma Sci 1997; 25:1066-72; http://dx.doi.org/10.1109/27.649627. Bouyer E, Girzhofer F, Boulou MI. The suspension plasma spraying of bioceramics by induction plasma. JOM 1997; 49:58-62; http://dx.doi.org/10.1007/BF02915483. Palma JI, Malinowski HJ, Smith WE. Tablet granulations of spherical hydroxyapatite powders: Their synthesis, application and characterisation. Clin Mater 1989; 4:319-27; http://dx.doi.org/10.1007/BF02239234. Sato S, Asai H, Sato Y, Fujii K, Kato H. Characteristics of a composite material for bone tissue regeneration. J Biomed Mater Res 2000; 52:1152-9; http://dx.doi.org/10.1002/1097-4636(200009)52:4<1152::AID-JBMR7>3.0.CO;2-K. Ebeling J, Taylor C, Ahrens C, Cardenas C, et al. Charaterisation and formation of a novel bioceramic hydroxyapatite composite. J Biomed Mater Res B Appl Biomater 2012; 99:311-20; http://dx.doi.org/10.1002/jbm.b.32196. Khor KA, Cheang P. Plasma sprayed hydroxyapatite (HA) coatings produced with flame-sprayed powders. J Mater Proc Technol 1997; 69:235-7; http://dx.doi.org/10.1016/S0924-0136(96)00263-9. Khor KA, Cheang P, Wang Y. Plasma Spraying of Combustion Flame Spheroidized Hydroxyapatite (HA) Powders. J Therm Spr Techn 1998; 7:254-60; http://dx.doi.org/10.1016/S0965-9477(97)031007. Koesh SWK, Khor KA, Cheang P. Characterisation of hydroxyapatite (HA) powders. J Mater Proc Technol 1999; 89:90-101; http://dx.doi.org/10.1016/S0924-3109(99)00161-8. Weng J, Wang M, Chen J. Plasma-sprayed calcium phosphate particles with high bioactivity and their use in bioactive scaffolds. Biomaterials 2002; 23:2623-9; PMID:12059011; http://dx.doi.org/10.1016/S0142-9612(01)00393-3. Hollister SJ. Porous scaffold design for tissue engineering. Nat Mater 2015; 4:518-24; PMID:26015400; http://dx.doi.org/10.1038/nmat1421.
112. Kandori K, Noguchi Y, Fukusumi M, Morisada Y.
111. Qiang Fu, Rahaman MN, Zhou N, Huang W, Wang.
110. Aizawa M, Terado T, Howell FS, Itatani K. Preparation
109. Galea LG, Bohner M, Lemaître J, Kohler T, Müller.
108. Nomura S, Tsuru K, Matsuya S, Takahashi I, Ishikawa.
106. Moseke C, Bayer C, Vorndran E, Barralet JE, Groll J,
105. Andrianjatovo H, Jose F, Lemaître J. Effect of beta-
104. Shi J, Chen L. Formation of calcium phosphates
103. Teng S, Shi J, Chen L. Formation of calcium phosphates
102. Butscher A, Bohner M, Hofmann S, Gauckler L, Müller.
101. Gbureck U, Hölzel T, Doillon CJ, Müller FA, Barralet.
100. Gbureck U, Hölzel T, Doillon CJ, Müller FA, Barralet.
99. Butscher A, Bohner M, Hofmann S, Gauckler L, Müller.
98. Nomura S, Tsuru K, Matsuya S, Takahashi I, Ishikawa.
97. 4-34.85; http://dx.doi.org/10.1080/01483918608077803.
96. 2912; 2012; 8:373-85; http://dx.doi.org/10.1177/0885328207081350.
95. 328-05; http://dx.doi.org/10.1016/j.colsurfb.2006.03.005.
94. 2011; 22:579-70; http://dx.doi.org/10.1080/01483918.2011.1030217.
93. 305:1587-94; http://dx.doi.org/10.1016/j.ceramicmaterial.2008.04.011.
92. Kazuwa M, Terado T, Howell FS, Itatani K. Preparation of spherical apatite particles by the homogeneous precipitation method in the presence of magnesium ions and their ion-exchange properties. Mater Res Bull 2008; 43:573-80; http://dx.doi.org/10.1016/j.materresbull.2008.03.005.
91. Shi J, Chen L. Formation of calcium phosphates in gelatin with a novel diffusion system. Colloids Surf B Biointerfaces 2006; 44:373-80; http://dx.doi.org/10.1016/j.colsurfb.2006.03.005.
90. Gbureck U, Hölzel T, Doillon CJ, Müller FA, Barralet.
89. Shi J, Chen L. Formation of calcium phosphates in gelatin with a novel diffusion system. Colloids Surf B Biointerfaces 2006; 44:373-80; http://dx.doi.org/10.1016/j.colsurfb.2006.03.005.
88. Shi J, Chen L. Formation of calcium phosphates in gelatin with a novel diffusion system. Colloids Surf B Biointerfaces 2006; 44:373-80; http://dx.doi.org/10.1016/j.colsurfb.2006.03.005.
87. Shi J, Chen L. Formation of calcium phosphates in gelatin with a novel diffusion system. Colloids Surf B Biointerfaces 2006; 44:373-80; http://dx.doi.org/10.1016/j.colsurfb.2006.03.005.
86. Shi J, Chen L. Formation of calcium phosphates in gelatin with a novel diffusion system. Colloids Surf B Biointerfaces 2006; 44:373-80; http://dx.doi.org/10.1016/j.colsurfb.2006.03.005.
85. Shi J, Chen L. Formation of calcium phosphates in gelatin with a novel diffusion system. Colloids Surf B Biointerfaces 2006; 44:373-80; http://dx.doi.org/10.1016/j.colsurfb.2006.03.005.
84. Shi J, Chen L. Formation of calcium phosphates in gelatin with a novel diffusion system. Colloids Surf B Biointerfaces 2006; 44:373-80; http://dx.doi.org/10.1016/j.colsurfb.2006.03.005.
83. Shi J, Chen L. Formation of calcium phosphates in gelatin with a novel diffusion system. Colloids Surf B Biointerfaces 2006; 44:373-80; http://dx.doi.org/10.1016/j.colsurfb.2006.03.005.
82. Shi J, Chen L. Formation of calcium phosphates in gelatin with a novel diffusion system. Colloids Surf B Biointerfaces 2006; 44:373-80; http://dx.doi.org/10.1016/j.colsurfb.2006.03.005.
81. Shi J, Chen L. Formation of calcium phosphates in gelatin with a novel diffusion system. Colloids Surf B Biointerfaces 2006; 44:373-80; http://dx.doi.org/10.1016/j.colsurfb.2006.03.005.
80. Shi J, Chen L. Formation of calcium phosphates in gelatin with a novel diffusion system. Colloids Surf B Biointerfaces 2006; 44:373-80; http://dx.doi.org/10.1016/j.colsurfb.2006.03.005.
