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Manufacture of hydroxyapatite beads for medical applications

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Abstract

Dense, microporous and macroporous hydroxyapatite spherical granules for medical applications were prepared by a new manufacturing procedure. This process is related to a lost wax method and yields beads with a fully controlled porosity, in terms of shape and pore size.

A $CaCO_3$ scaffold exhibiting controlled size spherical cavities is impregnated with an aqueous hydroxyapatite suspension. After drying in a plaster mould, a thermal treatment destroys the carbonate scaffold and makes it possible to release the HA beads. By this process, HA beads with diameters in the range of 300 μm to 3 mm are obtained. A post-thermal treatment of the beads allows the ceramic to be densified at various densification levels.

Porous ceramics make it possible to functionalise the hydroxyapatite granules by loading the structure with biologically active substances. Drug releasing kinetics in continuous distilled water flow were analysed as a function of time and ceramic porosity. Spheres continue to release the biological molecules for more than 5 days.

Keywords: Biomedical applications; Hydroxyapatite; Porosity; Shaping

1. Introduction

Bioceramic materials are widely used to repair and reconstruct damaged parts of the human skeleton especially as bone substitutes in the filling of bone defects.[1,2] Calcium phosphate ceramics and in particular hydroxyapatite (HA) and tricalcium phosphate β (β-TCP)[3–5] have received great attention for clinical practice. Because of their excellent biocompatibility, bioactivity and osteoconduction, these compounds are increasingly used as bone substitute materials. These materials are available in the form of injectable cements, granules or macroporous blocks.

Granules have been generally selected for classical bone filling.[6,7] However, calcium phosphate granules present irregular shapes and do not allow for optimal filling of a bone cavity or defect. Adjunction of a binding agent such as fibrin glue may stabilize the granules in the implantation site and produce a composite that can be moulded into the defect without empty spaces.[8,9]

Many processing routes have been used for the fabrication of porous hydroxyapatite granules, such as hydrothermal conversion of natural corals[10] and crushing of sintered blocks, granulation by vibration and rolling,[11] dripping procedure,[12] casting in plaster mould,[13] emulsion methods[14,15] and the spray-drying process.[16] However, these processes do not yield granules with optimal properties for medical applications. In the present work, a new manufacturing process has been developed to create HA granules with controlled shape, size and porosity. This process is similar to the lost wax process consisting in building a ceramic mould or carapace around a model part in wax or resin. In this work, the model part is constituted of calibrated PMMA balls and the carapace ceramic is calcium carbonate. The ceramic mould is emptied of the model part by using a thermal treatment. Because the plastic balls disappear, it becomes a "lost wax" method as the name of the process implied. The resulting empty space left by the lost resin thus forms spherical cavities shaped just like the balls. Cavities inside the carapace are then filled by an aqueous slurry of hydroxyapatite powder. When the ceramic is solidified, the carapace is destroyed to obtain the ceramic part, in this case hydroxyapatite beads (Fig. 1).

In the orthopaedic field, porous granules of HA can be used as drug delivery carriers, such as growth factors, anticancer

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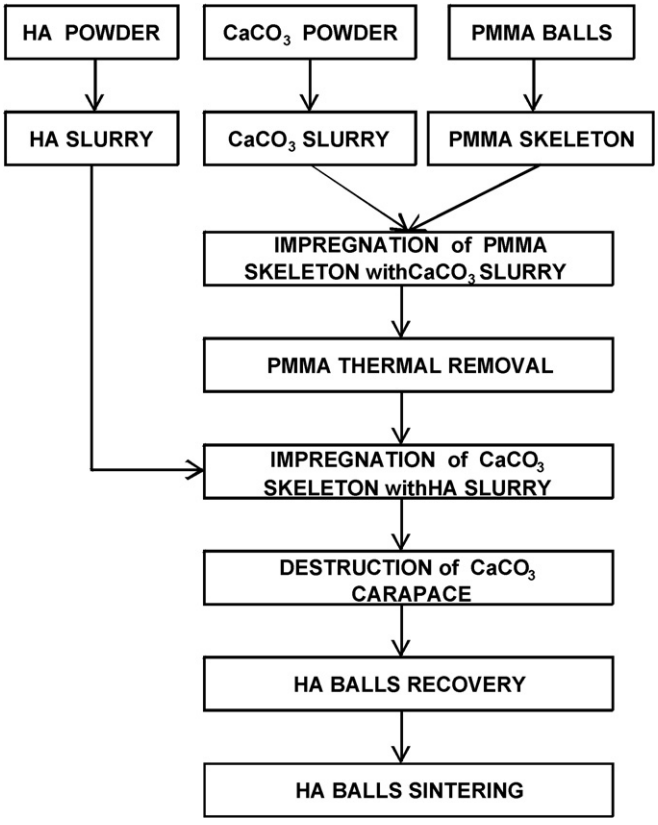

Fig. 1. Elaboration stages of HA balls.

drugs or antibiotic agents.[17] These drug delivery carriers allow an increasing of the local concentration of these bioactive substances in the implantation site with a progressive release. The drug concentration is maintained at a desired level for long periods of time without reaching a toxic level or dropping below the minimum effective level.[18] The effectiveness of these biological agents strongly increases in bone sites where vascularization is low. Among these products, the gentamicin[19–21] antibiotic is frequently used for its prophylactic action. This antibiotic prevents bacteria colonization responsable for infections and post-operative osteomyelitis. In this study, the drug loading and release rates in the as-prepared HA porous beads are tested with gentamicin antibiotic.

The topic of this paper is to present the fabrication technique used to form two types of beads: dense or microporous HA beads and macroporous HA beads and to present the first results of gentamicin impregnation in microporous beads.

2. Raw materials and experimental techniques

2.1. Preparation of the organic scaffold

An organic scaffold is build from polymer balls, calibrated and welded together. The PMMA (Diakon^{TM} Ineos Acrylics Holland, Saluc Belgium) is used to construct the polymeric frame. Spherical shape of this agent allows the building of a frame with controlled dimension and morphology. Organic frames were formed with various ball sizes obtained by mechanical sieving and in particular for diameters in the range of 300–400, 400–500 and 600–700 μm and with calibrated balls with diameters equal, respectively, to 2 and 3 mm.

Table 1
Physical and chemical properties of $CaCO_3$ powder

Specific surface area (m^2/g)	3.3
Mean particle size (μm)	3
Density	2.7
Chemical purity (% $CaCO_3$)	>99

The organic scaffold is made by stacking the PMMA balls. The bridging between polymeric balls is obtained by a chemical treatment[22] using acetone (RPE 99.8% Carlo Erba, France) which exhibits a dissolution chemical action with respect to the PMMA. When this solvent is poured on the ball pile, a slow dissolution appears at the ball surface and induces an overlapping between the individual bodies. This movement leads to the formation of necks between the PMMA balls and a significant shrinkage of the ball pile. Measurement of the dimensional variation of the organic frame allows the ball neck size control.

2.2. Preparation of calcium carbonate slurry and impregnation of the organic scaffold by calcium carbonate

The as-prepared organic skeleton is then impregnated by the calcium carbonate suspension in order to fill voids between the polymeric PMMA particles. Physical and chemical characteristics of the $CaCO_3$ powder (Mikhart 2, Provençale S.A, France) are shown in Table 1.

$CaCO_3$ aqueous slurries were prepared with 64 wt.% dry matter. Slurry defloculation is assured by a commercial organic agent (Darvan C, R.t.Vanderbilt.Co.) in an amount equal to 1.5 wt.% of $CaCO_3$ content. A quantity of organic binder (4 wt.% of $CaCO_3$ content, Duramax B1001, Rohm and Haas) was added to ensure a consolidation of green material during the debinding treatment. Slip preparation is carried out using a planetary ball mill with agate container and balls. Milling duration is fixed to 1 h with a rotation speed equal to 180 rev/min.

The organic skeleton is placed in a plaster mould and the $CaCO_3$ slurry is poured on these PMMA balls. After sample drying in a plaster mould, a debinding treatment eliminates the porogen agent (PMMA) and creates the macroporosity within the ceramic. The debinding cycle was optimised by PMMA thermogravimetric analysis (Setaram SETSYS 16/18).

2.3. Impregnation of calcium carbonate scaffold by HA powders

2.3.1. (i) Synthesis of HA powder

Stoichiometric HA powder was prepared by aqueous precipitation method using diammonium phosphate $(NH_4)_2HPO_4$ (Carlo Erba, France) and a calcium nitrate Ca $(NO_3)_24H_2O$ (Brenntag, France) solutions.

The solution pH was adjusted at a constant value of 11 by a continuous addition of ammonium hydroxide. Temperature was fixed to 50 °C and the solution was matured for 20 h. After ripening, the slurry was filtered and the precipitate was dried at

80 °C. After calcination at 900 °C, powder was ground to break up agglomerates formed during the calcination. This grinding step was carried out by ball milling with HDPE milling jar and Y-TZP grinding for 48 h. After this treatment, the specific surface area of ground powders recorded by the BET method (Micromeritics, Flow Sorb 3) was equal to 5.2 m^2/g.

HA aqueous slurry was prepared under the same experimental conditions as those for the $CaCO_3$ suspension. Powder concentration was equal to 75 wt.% and dispersion powder was assured by Darvan C in amount equal to 1.5 wt.% of HA content.

2.3.2. (ii) Elimination of calcium carbonate mould and recovery of HA balls

After thermal degradation of ceramic carapace, the HA balls are collected.

The characteristics of the as-obtained porous beads are determined by mercury porosimetry (Poresizer 9310, Coultronics).

2.4. Delivery of biological agent

The microporous HA beads are impregnated with the broad-spectrum antibiotic Gentamicin. HA beads with 20% of open porosity and diameter in the range of 600–700 μm are vacuum impregnated with a solution of gentamicin (20 mg/g of HA), then dried at 37 °C for 24 h.The effectiveness of the impregnation was evaluated by thermogravimetric analysis.

1 g of impregnated beads of was placed in a 2-cm^3 flow cell thermostated by a digital dry bath (Fisher scientific FB15103). The fluid circulation was performed by a high precision tubing pump with planetary drive (ISMATEC IPC-N ISM936). Distilled water was used as solvent with a flow volume of 6 cm^3/min. Gentamicin release evaluation was performed at 255 nm wavelength on a UV–vis spectrophotometer (SHIMADZU UV-2500 PC). Data were recorded on specific spectroscopy software (UV.Prob 2.21).

3. Results

3.1. Manufacturing of $CaCO_3$ ceramic mould

Bridging between PMMA beads yields a compact which will be impregnated by the $CaCO_3$ suspension. Thermal elimination of this polymeric agent creates interconnected cavities.

These interconnections constitute a continuous supply network between these spherical chambers. Interconnection size is adjusted to achieve a sufficient cohesion between the PMMA balls and to allow an easy handling of the organic frame.

An empirical study conducted for each bead grain size repartition provided the neck sizes presented in Table 2. This work has been described in a previous paper.[22]

Table 2
Interconnection size for different bead sizes

Ball size (mm)	0.3–0.4	0.4–0.5	0.6–0.7	2	3
Neck size (μm)	85	100	150	300	400

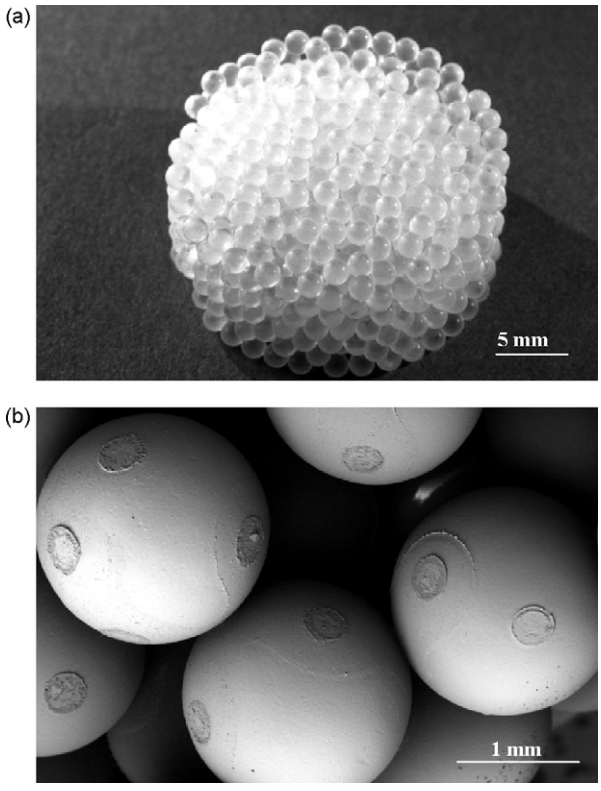

Fig. 2. Organic frame (a) PMMA block and (b) necks between beads.

Fig. 2a represents a macroscopic view (Reflex Canon EOS 300 D) of PMMA block with a bead diameter equal to 2 mm, obtained after a chemical treatment. Fig. 2b presents pictures obtained by SEM observations of block fracture faces showing the neck size between PMMA balls.

The as-prepared organic skeleton is then impregnated by the calcium carbonate slurry in order to fill the voids between PMMA particles. Fig. 3a and b respectively represent a macroscopic view of a polished block of PMMA ball ($\Phi = 2$ mm) impregnated with $CaCO_3$ slurry and fracture faces of this sample.

The PMMA elimination is carried out by a thermal treatment at low temperature. A thermogravimetric analysis shows that a heating to 220 °C for 20 h followed by a dwell at 250 °C for 10 h makes it possible to eliminate 97% of the organic compound (Fig. 4). The residual organic (3%) ensures a sufficient and necessary mechanical strength for the handling of samples during the later stages.

After this debinding treatment, the sample is constituted of macropores with a size equal to the size of the used PMMA balls. These cavities are interconnected. Fig. 5a and b respectively represent a macroscopic view of polished $CaCO_3$ scaffold and its fracture faces.

3.2. Manufacturing of dense or microporous HA beads

The calcium carbonate scaffold is impregnated by aqueous suspensions of hydroxyapatite in order to fill the cavities left by the PMMA balls. This operation is performed into a plaster mould in order to allow the drying of the part.

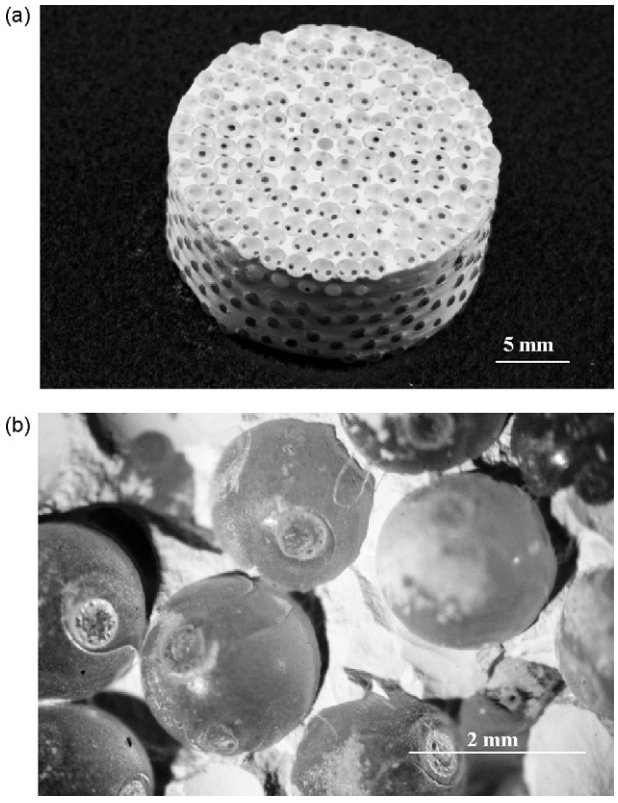

Fig. 3. PMMA skeleton impregnated with $CaCO_3$ slurry: (a) PMMA block and (b) fracture facies.

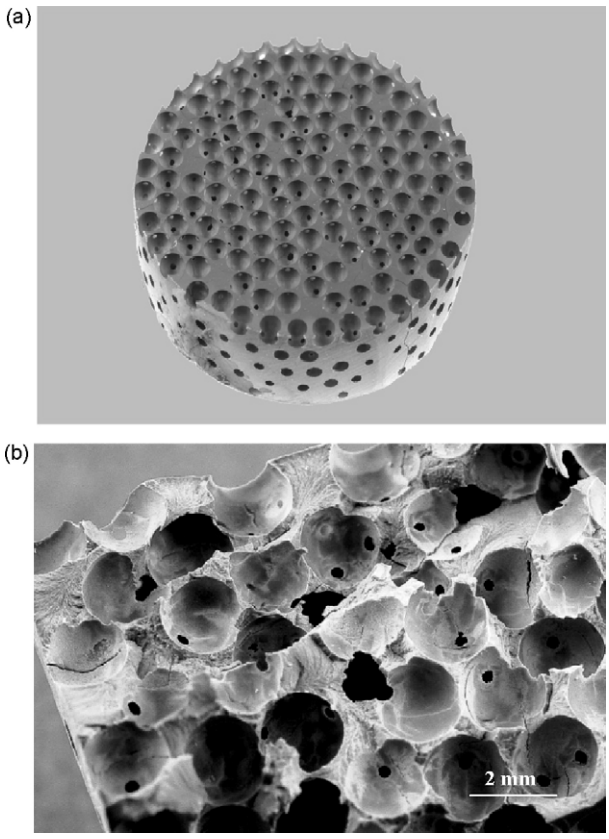

Fig. 5. $CaCO_3$ Scaffold: (a) block and (b) fracture facies.

Fig. 6a represents a macroscopic view of the polished $CaCO_3$ mould filled with hydroxyapatite after drying. Interconnections between cavities allow the feeding and optimal filling of the $CaCO_3$ scaffold by the hydroxyapatite suspension (Fig. 6b) and moulding of HA beads, as shown by the fracture surfaces of the sample (Fig. 6c).

After filling the $CaCO_3$ scaffold with the hydroxyapatite powder, the material is heated up to 1000 °C to transform the calcium carbonate to lime, according to the following chemical reaction; $CaCO_3 \rightarrow CaO + CO_2$.

Thermogravimetric analysis conducted on the $CaCO_3$ raw powder with a heating rate of 0.5 °/min shows that this transformation occurs between 590 and 650 °C (Fig. 7). However, a higher temperature is applied to induce a pre-sintering of the HA powder and a consolidation of the ceramic balls in order to resist during the demolition of the calcium carbonate mold.

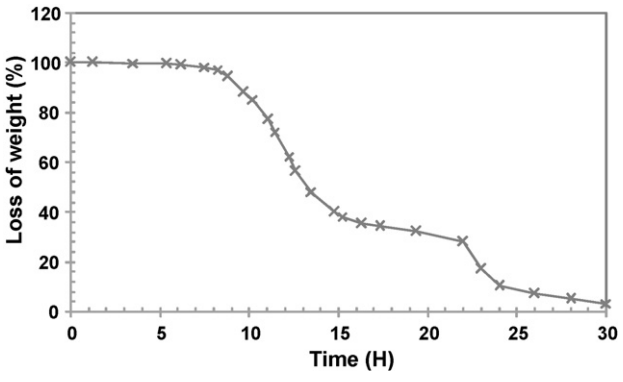

Fig. 4. Thermogravimetric analysis of PMMA beads.

During cooling, the samples undergo a water quenching from 200 °C to transform the lime into calcium hydroxide. This reaction is accompanied by a significant swelling of the structure causing the $CaCO_3$ ceramic disintegration and release of the HA beads. After this step, HA balls and Ca $(OH)_2$ powder are separated by mechanical sieving.

Fig. 8a–c represents a macroscopic view of HA beads obtained using a $CaCO_3$ scaffold made from various PMMA balls diameters in the range of 300–400, and 600–700 μm and 3 mm, respectively.

In order to obtain different porosity ranges, beads are sintered at different temperatures: 1275, 1150 and 1100 °C with a time dwell equal to 3 h. Sintering at the highest temperature makes it possible to obtain 97.5% as relative density. On contrary, the lowest temperatures (1150 and 1100 °C) offer porous beads characterized by an micronic open porosity volume equal to 20 and 42%, respectively.

3.3. Manufacturing of macroporous HA beads

The above described technique enables the manufacture of dense or microporous balls of HA. Low sintering temperatures achieve a maximum volume of microporosity around 40%. Above this pore volume, the sample does not present sufficient cohesiveness and mechanical strength for the listed applications.

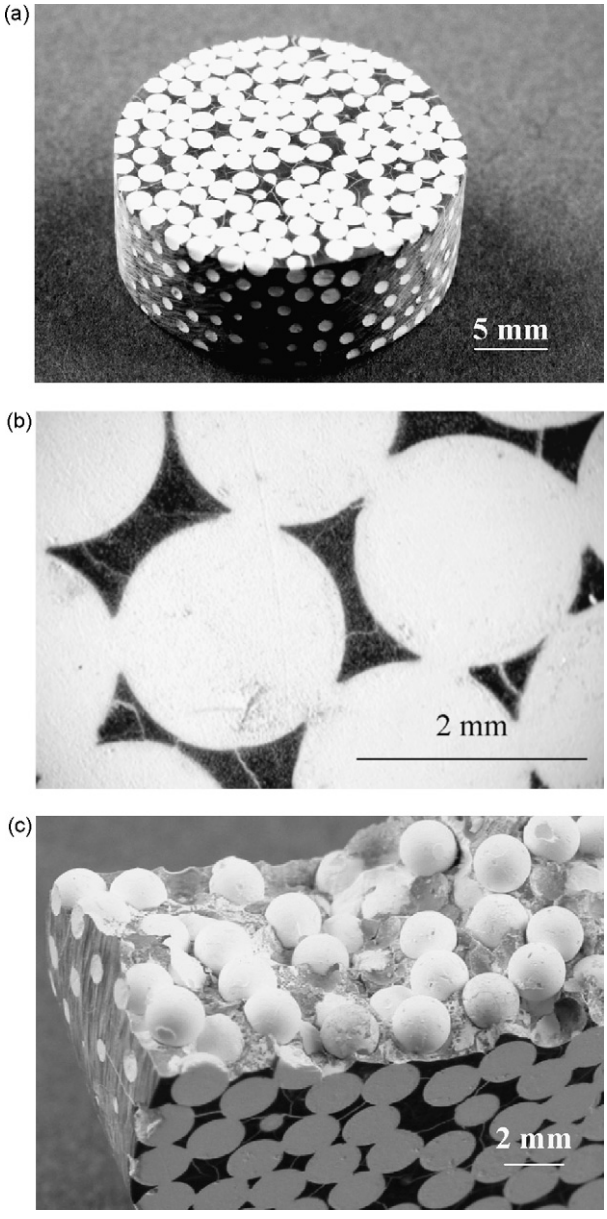

Fig. 6. $CaCO_3$ mould filled with hydroxyapatite.

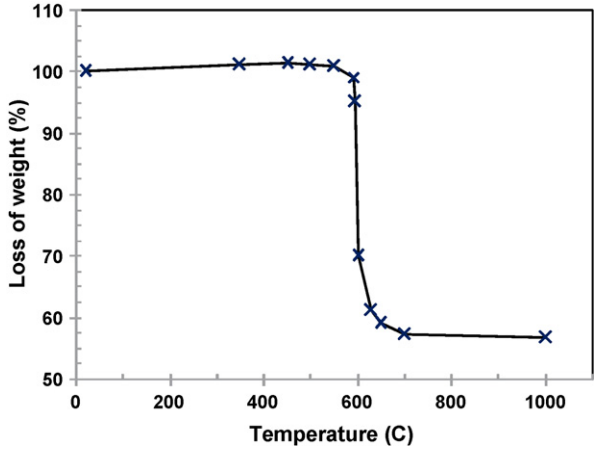

Fig. 7. Thermogravimetric analysis of $CaCO_3$.

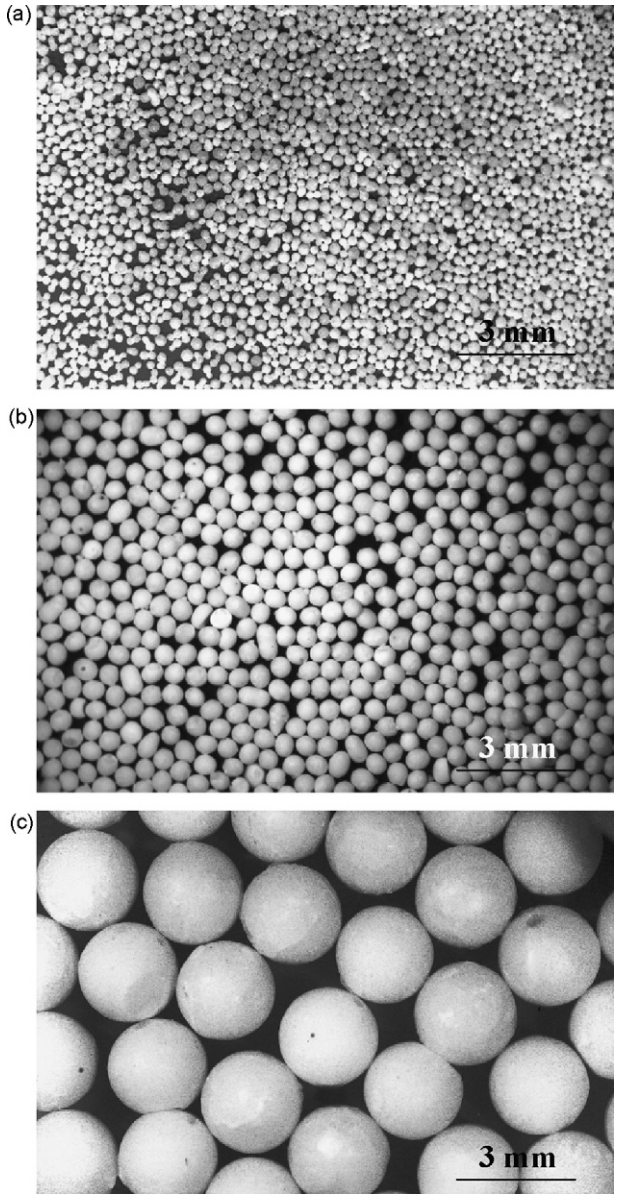

Fig. 8. Hydroxyapatite beads obtained with $CaCO_3$ scaffold fabricated using PMMA balls equal (a) 300–400 μm and (b) 600–700 μm, 3 mm.

A variation on the technique presented in the previous section can be used to prepare macroporous balls exhibiting higher porous volumes up to 70 and 80%. These macroporous balls are obtained by filling the cavities of $CaCO_3$ scaffold before the HA slurry impregnation with PMMA balls. The diameter of these PMMA balls should be lower than the interconnection diameter between the spherical cavities. As a result, these tests were performed with diameter beads of PMMA ranging from 80 to 100 μm and on a $CaCO_3$ scaffold obtained with diameter beads equal to 2 and 3 mm which have interconnection diameters equal to 300 and 400 μm, respectively.

The filling of the $CaCO_3$ scaffold cavities by the small PMMA balls is achieved by using a vibrating table to get a maximum filling. After this operation, the PMMA balls are impregnated by a kenotic solution for a few minutes to perform bridging between the PMMA balls. Fig. 9 represents the fracture

Fig. 9. Fracture facies of $CaCO_3$ scaffold filled with PMMA beads.

faces of a $CaCO_3$ scaffold filled with PMMA necked balls. The $CaCO_3$ scaffold filled with PMMA beads is then impregnated with the HA suspension. A thermal treatment at 220 °C for 20 h removes a significant part of the polymeric agent from inside the resulting HA spheres. This debinding treatment is followed by a thermal treatment to 1000 °C to transform the $CaCO_3$ to CaO and consolidate the HA macroporous balls, a water quenching and finally a screening to recover the macroporous balls.

Fig. 10 represents a macroscopic view of the as-obtained macroporous beads. These HA beads are constituted of interconnected macropores of about 80 μm size (Fig. 11).

3.4. Application of HA beads for biological agent delivery

We have seen that the process makes it possible to obtain various kinds of spheres like dense or microporous with different porosity volumes. The microporosity allows a ceramic functionalization by loading the porosity with various biological active substances. Thermogravimetric analysis carried out on HA beads impregnated with gentamicin (Fig. 12) shows an initial weight loss of 0.4% between 20 and 200 °C which corresponds to residual moisture. A second weight loss appears from 230 °C, and continues up to 600 °C. This behavior is induced by the gentamicin combustion, with a weight loss (2%) equal to

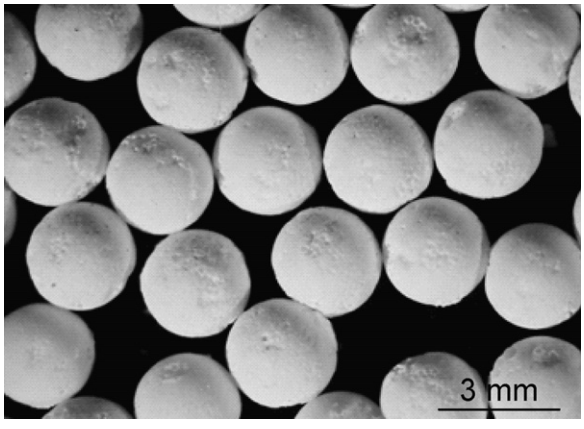

Fig. 10. HA macroporous beads.

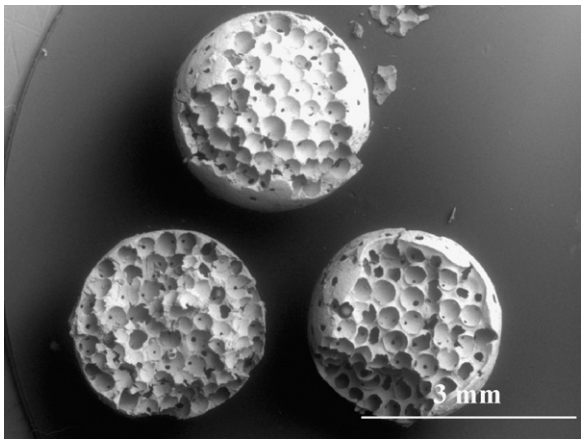

Fig. 11. Fracture facies of HA macroporous beads.

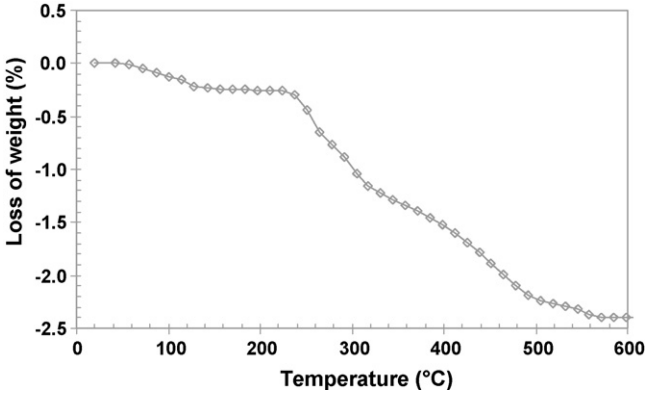

Fig. 12. Thermogravimetric analysis of Hydroxyapatite beads filled with gentamicine.

the gentamicin weight introduced within the HA beads. So, the whole gentamicin amount used for impregnation is completely taken up by the granules.

To evaluate the drug release rate in close physiological environment conditions, the gentamicin-loaded granules were placed in a continuous distilled water flow (6 ml/h). Fig. 13 shows the gentamicin amount released versus time. For the chosen structure (20% of open porosity and bead diameter in the range of 600–700 μm), more than 85% of gentamicin was released within

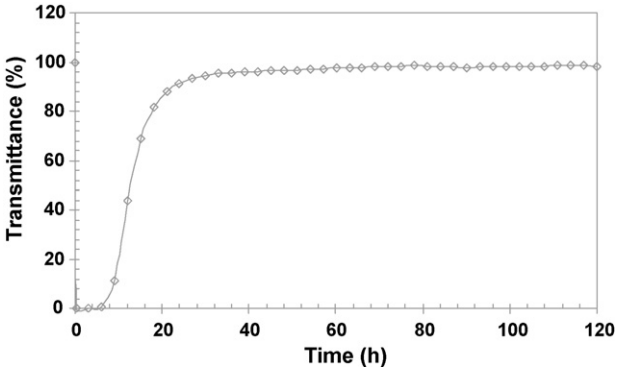

Fig. 13. Gentamicine release profile of HA beads.

24 h. After this period, the released amount decreases over 5 days.

This first experiment reveals that microporous HA microspheres can be used as a matrix for a local drug delivery system to prevent and treat infections associated to bone implants. The drug release quantity and duration can be controlled by the morphology and microstructure of the HA beads and in particular by the porous volume in order to obtain a drug delivery system according with the therapeutic dosage.

4. Conclusion

During this study, a new process for fabrication of a drug delivery system combining an osteoconductive material and a bioactive agent has been developed. This new process based on the wax loss method makes it possible to control the various architectural parameters such as, spherical shape, size and porosity of the drug containers. Beads with a diameter ranging from 300 μm to 3 mm with various open pore volumes (0, 20, 40%) are obtained by controlling the sintering temperature. Microporous beads were impregnated by an anti-infectious biological agent: gentamicine. Drug release experiment showed a gentamicin release from the HA beads for up to 5 days.

Macroporous beads with porous volume of 70% were also produced by adapting the method to introduce a macroporosity. These beads are constituted of spherical interconnected macropores with dimensions equal to 80 μm. The high porosity volume of macroporous beads, compared with microporous beads, should allow storage of larger quantities of biological agents and increase therefore their effectiveness. Currently, impregnation tests of these macroporous beads with biodegradable polymers (polylactic acid, polyglycolic acid, polylactide-*co*-glycolide) mixtures and biological agents are in progress.

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