ABSTRACT

Highly porous ZrO₂ scaffolds fabricated by the replication method were coated with fluorapatite (FA). The FA coating was obtained by infiltrating the ZrO₂ scaffolds with stabilized aqueous FA slips having different viscosity values: ~1.0, 2.2 and 5.0 mPa.s. The influence of the FA slip viscosity on the coating distribution and structure of the coated scaffolds was investigated. The FA slip with ~1.0 mPa.s viscosity did not lead to a continuous coating. The coating with FA slips having 2.2 mPa.s viscosity was uniformly distributed along the strut surfaces and formed a thin layer inside the macropores. On the contrary, coating with FA slips of 5.0 mPa.s viscosity resulted in thicker FA layers which strongly reduced the pore sizes and increased the struts thickness of the original ZrO₂ scaffolds. The porous structure obtained after coating with slips of 2.2 mPa.s viscosity is expected to be optimum for tissue ingrowth and bone formation.

Key-words: ZrO₂ scaffolds, fluorapatite coating, fluorapatite slip viscosity, uncoated and coated scaffolds structure.

INTRODUCTION

Fluorapatite has been used in form of particles or granules as bone substitute materials in orthopedic and dental applications (1,2). The main drawback still impairing the use of fluorapatite in load-bearing applications is their intrinsic brittleness. This aspect becomes even more critical when these materials are used in form of highly porous scaffolds, e. g. for bone tissue engineering applications, making
very difficult their handling during surgical procedures and facing a high probability of failure after implantation. Therefore, the design of scaffolds for bone regeneration should be based on the criterion of optimizing their mechanical strength and biological compatibility.

3 mol% yttria-partially stabilized zirconia (Y-TZP) is used as a structural ceramic owing to its excellent mechanical properties (3). In this context, a porous ZrO₂ scaffold was chosen as a framework and the fluorapatite was used as a coating layer to enhance the biocompatibility and osteoconductivity. The production of a highly porous coated ZrO₂ scaffold is one of the crucial requirements in order to ensure exchange of ions between the scaffold and the surrounding biological environment, migration of cells and tissue formation (4). The FA coating was achieved by infiltrating the ZrO₂ scaffolds with an aqueous well-stabilized FA slip with poly(vinyl)alcohol (PVA).

In order to achieve a continuous thin film of fluorapatite uniformly distributed along the strut surfaces and inside the macropores of the ZrO₂ scaffold, the rheological behaviour and viscosity of aqueous FA slips should be investigated. Successful colloidal processing of fine ceramic powders requires accurate control of both rheological properties and the state of the dispersion. Anionic polyelectrolytes such as ammonium polyacrylate (NH₄PA) are commonly used as dispersant of ceramic powders in aqueous media (5). The binder such as PVA provides strong adhesion between the ceramic particles and the substrate, preventing the powder from detaching off the substrate during thermal treatment.

In this study, porous ZrO₂ scaffolds fabricated by the replication method were coated with FA aqueous slips stabilized with NH₄PA having different viscosity values. The influence of the FA slip viscosity on the coating distribution and structure of the coated scaffolds was investigated. The structures of uncoated and coated ZrO₂ scaffolds were characterized.

EXPERIMENTAL PROCEDURE

Raw materials and processing

A commercial 3 mol% yttria-partially stabilized zirconia (Y-TZP) (Saint-Gobain ZirPro, China, d₅₀= 0.64 µm) was used to produce the scaffolds. The Ca₃(PO₄)₂ (Fluka, Germany) and CaF₂ (Sigma–Aldrich, Ireland) powders were mixed in stoichiometric ratio and calcined 3 h at 1000 °C. Then, the powder was milled in an
attrition mill, washed with distilled water and dried at 100 °C. This powder subsequently referred as FA was used to prepare the suspensions for dip coating. A commercial NH₄PA solution (Duramax D 3500, Rohm & Haas, Philadelphia PA) and a 9 wt% PVA solution were used as deflocculant and binder, respectively. A fully reticulated polyurethane foam in a shape of disc (12 mm in diameter and 3mm in thickness) was used as sacrificial template for the foam replication method. For the impregnation of the polyurethane foams, aqueous Y-TZP suspensions with a solid loading in the range 35-44 wt% were prepared by deagglomeration of the powder in distilled water with 0.11 wt% NH₄PA using an ultrasonic bath. Subsequent to this, 6 wt% PVA was added to the slurry, followed by additional stirring. The pH of the suspensions was adjusted to 9.0 with ammonia (25 wt%). Discs of polyurethane foams were immersed in the above prepared slurry and centrifuged for 5 min at 2000 rpm. The sponges were then dried at 100 °C for 24 h. The obtained bodies were heated to burn out the sponge and binder at 800 °C for 5 h at a heating rate of 1 °C/min; finally they were sintered at 1500 °C for 2 h. The 5, 10 and 20wt% aqueous FA slurries with 0.6wt% NH₄PA and 5wt% PVA were prepared for the coating process. The fabricated Y-TZP scaffolds were immersed in the FA slurries for 2 sec, then manually withdrawn from the suspension at a constant velocity and dried at 100 °C for 24 h. The coated ZrO₂ scaffolds were sintered at 1100 °C for 2h.

**Characterization techniques**

Steady state flow curves of FA slips were performed by measuring the steady shear stress value as a function of shear rate in the range of 0.5 to 542 s⁻¹ using a concentric cylinder viscometer (Haake VT550, Germany) and a sensor system NV at 25 °C. As soon as stationary conditions were reached at each shear rate, the shear rate increased in steps up to the maximum value and then decreased. The density of the scaffolds (ρₛ) was determined from the mass and dimensions of the sintered bodies. The total porosity p was then calculated by:

\[ P = 1 - \frac{\rho_s}{\rho_z} = 1 - \rho_{\text{relative}} \]  \hspace{1cm} (A)

where \( \rho_z = 6.05 \text{ g/cm}^3 \) is the theoretical density of Y-TZP.

The total porosity of coated samples was calculated by the modified relationship:

\[ P = (1 - \frac{\rho_s}{\rho_z} - \frac{\rho_{\text{cm}}}{\rho_{\text{ct}}}) \times 100 \] \hspace{1cm} (B)

where \( \rho_{\text{cm}} \) is the coating density calculated for each sample as:

\[ \rho_{\text{cm}} = \frac{w_i - w_f}{V} \] \hspace{1cm} (C)
and \( w_f \) are respectively the weight of the scaffold before and after coating and \( V \) is the scaffold’s volume; \( \rho_{\text{ct}} \) is the theoretical coating density (\( \rho_{\text{fluorapatite}} = 2.3 \text{ g/cm}^3 \)).

The microstructure of the scaffolds was characterized using scanning electron microscopy (SEM) (JEOL, JCM-6000). A quantitative assessment of pores size and struts thickness distribution was carried out by SEM; in order to perform a reliable statistical sampling more than 250 cells were measured from different images. The coated scaffolds were polished (with a series of diamond pastes down to 1 \( \mu \)m) for microstructural observation by SEM.

RESULTS

Rheological properties

Figures 1a and 1b show the flow curves of shear stress versus shear rate and viscosity versus shear rate, respectively, for FA slips with different solid loading at pH 9.

![Figure 1: Flow curves of shear stress versus shear rate (a) and viscosity versus shear rate(b), for FA slips with 0.6 wt% NH₄PA, 5 wt% PVA with different solid loading at pH 9.](image)

The suspensions exhibited a nearly Newtonian behaviour, well-stabilized FA slips with low viscosity values were obtained for all the solid loading. We have previously studied the rheological behaviour of aqueous FA slips stabilized with NH₄PA (6), the isoelectric point (IEP) of the FA powder was at about pH 6. The FA exhibits a negative surface charge at pH > 6 and a positively one at pH values lower...
than the IEP. Fluorapatite surfaces are thought to consist of two distinct surface groups $\equiv\text{CaOH}_2^+$ and $\equiv\text{PO}^-$ (7); according to Chaïrat et al. (8), the $\equiv\text{CaOH}_2^+$ concentration decreases with increasing pH and at pH>9.5 the $\equiv\text{CaOH}^0$ sites predominate.

The addition of $\text{NH}_4\text{PA}$ shifts the IEP of FA towards a lower pH value of 5 (6). The ammonium polyacrylate dissociation according to the reaction:

$$\text{RCOONH}_4 \rightarrow \text{RCOO}^- + \text{NH}_4$$

begins at pH>3.5; at pH values ≥8.5 the polymer charge is negative with the degree of ionization approaching 1 (5). During the aqueous colloidal processing of the FA powder with 0.6 wt% $\text{NH}_4\text{PA}$, the pH of the suspension was adjusted at 9. At this pH, the RCOO$^-$ groups of the deflocculant were adsorbed at the positive $\equiv\text{CaOH}_2^+$ sites of the FA powder surface. The adsorption of the negatively charged polyelectrolyte increases the negative surface charge of the FA powder and consequently the electrostatic repulsion between particles. In addition, at pH 9 the electrostatic repulsion between the charged carboxylate groups impedes the accumulation of groups at the surface, the polyelectrolyte adsorbs in a stretched-out configuration which results in long-range steric interactions of the NH$_4$PA at the solid-liquid interface (5). Thus, the adsorbed molecules increased the electrosteric repulsion between particles, thereby low viscosity values were achieved (fig. 1).

We have recently determined (9) that the degree of slip dispersion of a ceramic powder at low solid loading (20 wt%) was nearly the same with and without PVA. Thus, the PVA did not affect the dispersion properties of the FA powder which were basically controlled by the dispersant added.

The slip viscosity markedly increased with increasing the solid content (fig. 1). As the FA content increased from 5 to 20 wt%, the viscosity of the slips increased from ~1.0 to 5.0 mPa.s, respectively. The increase in the solid loading forces the particles to approach each other and overlap their electrical double layer (electroviscous effect), thereby decreasing the electrostatic potential and consequently increasing the slip viscosity (10).

Scaffolds structure

Figure 2 shows the porosity change of the ZrO$_2$ scaffolds as a function of the aqueous ZrO$_2$ slip concentration. The porosity of the scaffolds increased from 80 to 93% with decreasing the ZrO$_2$ slip concentration from 44 to 35 wt%, respectively. The scaffolds
with an extremely high porosity of 93% were fragile to handle; on the other hand, some pores were observed to be partly blocked when the porosity of the ZrO$_2$ scaffold was reduced to 80%. Consequently, the scaffolds with 87%, prepared from 40 wt% slips, were selected to be coated with a fluorapatite surface layer. Based on these results, the scaffold porosity was controlled by changing the aqueous ZrO$_2$ slip concentration.

![Figure 2: Total porosity of the ZrO$_2$ scaffolds as a function of the aqueous ZrO$_2$ slip concentration.](image)

Figure 2: Total porosity of the ZrO$_2$ scaffolds as a function of the aqueous ZrO$_2$ slip concentration.

Figure 3a shows a SEM image of the ZrO$_2$ scaffold with 87% porosity. The ZrO$_2$ scaffold exhibited a well-developed open macropore structure; the majority of the pores were open, whereas only a few ones were blocked or closed. Thus, the ZrO$_2$ scaffold total porosity was nearly almost open or interconnected. As reported in several works dealing with scaffolds for bone replacement (11), the achievement of a highly open porous structure is one of the crucial requirements in order to ensure exchange of ions between the scaffold and the surrounding biological environment, migration of cells and tissue formation. A sufficient degree of open porosity is also beneficial for more effective penetration of the fluorapatite coating into the ZrO$_2$ scaffold structure.

Figures 3b, 3c and 3d show SEM images of the ZrO$_2$ scaffolds coated by FA slips having viscosity values of ~ 1.0, 2.2 and 5.0 mPa.s, respectively. The microstructure of the coated scaffolds showed the ZrO$_2$ strut surface (bright phase), the FA coating (grey phase) and the pores (dark phase). SEM images of coated scaffolds with slips of 5.0mPa.s viscosity, showing the FA layer inside the pores were observed in Figs. 3e, 3f.
Figure 3: SEM images of different ZrO₂ scaffolds: (a) uncoated, (b) coated with slip of ~1.0 mPa.s viscosity, (c) coated with slip of 2.2 mPa.s viscosity, (d, e, f) coated with slip of 5.0 mPa.s viscosity. ZrO₂ (bright phase), FA coating (grey phase), pores (dark phase).

Figure 4 shows the volume percent of the scaffold porosity occupied by FA and the amount of FA introduced into the scaffold per cm³ as a function of the slip viscosity. Both, the vol% of FA and the mg FA/cm³ introduced into the ZrO₂ scaffolds, linearly increased with increasing the slip viscosity. A low amount of FA (2.7 mgFA/cm³) was incorporated into the scaffold after coating with slips of ~1.0 mPa.s viscosity,
resulting in a negligible reduction of the scaffold total porosity. As the dip-coating slip viscosity increased to ≥2.2 mPa.s, a greater amount of FA was introduced per cm³ and the volume percent of the scaffold porosity occupied by FA increased. Nearly 3 vol% of the porosity was occupied by FA in the scaffold coated with slips of 5.0 mPa.s viscosity, in comparison with 1 vol% in the scaffolds coated with slips having a viscosity value of 2.2 mPa.s (fig. 4).

Figure 4: Volume percent of the scaffold porosity occupied by FA and amount of FA introduced into the scaffold per cm³ as a function of the slip viscosity.

The coating of the ZrO₂ scaffolds with FA slips of ~ 1.0mPa.s viscosity produced an inhomogeneous film along the strut surfaces (fig. 3b). The amount of FA introduced into the ZrO₂ scaffold after the coating procedure was almost negligible (fig.4), indicating a non uniform distribution of the coating in the structure. The low viscosity of the aqueous 5 wt% slip (fig. 1) caused a rapid flow after infiltration and avoided an homogeneous coating. Thus, this slurry did not present enough viscosity to remain in the ZrO₂ scaffold during drying. An adequate slip to produce a coating must be fluid enough to penetrate into the structure and also presents enough viscosity to remain in it (12). As a consequence, the 5 wt% dip-coating slurry did not lead to a continuous FA film along the strut network and the macropores remained uncoated. The well-dispersed FA slip with 2.2 mPa.s viscosity enabled a greater incorporation of FA into the scaffold (fig. 4), resulting in a coating uniformly distributed along the strut surfaces and inside the macropores forming a thin FA layer (fig.3c); the thickness of the FA layer adhered was about 10-20 µm. This thin FA layer did not change the polygonal shape of the ZrO₂ scaffold macropores (fig. 3c).
The higher viscosity value of the 20 wt% FA slip with respect to that of 10 wt% (fig.1) formed an homogeneous coating along the strut network and produced thicker adhered layers (60-80 µm) inside the pores (figs. 3d, 3e and 3f). The pores with small sizes were completed closed by FA (fig. 3f), whereas the ones with large sizes were partially blocked (fig.3e). Thus, a significant volume percent of the ZrO₂ scaffold porosity was occupied by the FA adhered layer, producing an important reduction of the scaffold pores volume and a change in the pores shape from polygonal (in the uncoated scaffold) to round or irregular (in the scaffold coated with slips of 5.0 mPa.s viscosity) (figs.3 and 4).

The pore size and strut thickness distributions of the ZrO₂ scaffolds before and after coating with slips of 1.0, 2.2 and 5.0 mPa.s viscosity are shown in figures 5a and 5b, respectively. A wide pore size distribution with pore sizes between 250 and 700 µm was found for uncoated ZrO₂ scaffolds; most of the pores had sizes ranging from 340 to 540 µm and the most frequent pore size was in the range 400-420 µm.

For the scaffolds coated with FA slips with lowest viscosity, a similar pore size distribution with respect to the uncoated ZrO₂ scaffold was found (fig. 5a). The most frequent pore size was in the range 400-420 µm. Besides, nearly the same strut thickness distribution was observed for the uncoated and coated ZrO₂ scaffolds with lowest viscosity slip.

The struts had thickness between 50 and 85 µm and the more frequent strut thickness was in the range 65-70 µm. Thus, the low amount of FA introduced into the
scaffold resulted in an inhomogeneous coating which did not reduce the porosity or increased the strut thickness of the original ZrO$_2$ scaffolds structure. The coating of the ZrO$_2$ scaffolds with slips of 2.2 mPa.s viscosity produced a slightly displacement of the more frequent pore size from 400-420 to 360-380 µm. In addition, a scarcely increased in the most frequent strut thickness from 65-70 to 70-75 µm was measured. In contrast, the pore size distribution of the ZrO$_2$ scaffold was significantly shifted to lower sizes after the coating with slips of 5.0 mPa.s viscosity. A strong reduction of the most frequent pore size from 400-420 to 220-240 µm occurred in accordance with the greater volume percent of the porosity occupied by FA (fig. 4). The strut thickness distribution was also shifted to significantly greater values after the coating; the most frequent strut thickness increased from 65-70 to 80-85 µm for uncoated and coated scaffolds with slips of 5.0 mPa.s viscosity, respectively. Thus, the coating with slips of 5.0 mPa.s viscosity affected the whole porous structure of the ZrO$_2$ scaffolds.

The porous structure obtained after coating with slips of 2.2 mPa.s viscosity, with a low reduction of the pore size by the FA layer and a continuous film distributed along the strut surfaces, is expected to be optimum for tissue ingrowth and bone formation. On the contrary, the greater amount of FA introduced into the scaffolds after coating with slips of 5.0 mPa.s viscosity resulted in thicker FA layers which strongly reduced the pore size and increased the strut thickness of the original ZrO$_2$ scaffolds. It was expected that the large amount of FA introduced in the scaffolds coated with slips of 5.0 mPa.s viscosity would certainly decrease its biological activity by reducing the pore volume and the pore size.

CONCLUSIONS

Three dimensional, highly porous, ZrO$_2$ scaffolds coated by fluorapatite were fabricated. The FA coating was achieved by infiltrating the ZrO$_2$ scaffold with stabilized aqueous FA slips having different viscosity values. The influence of the FA slip viscosity on the coating distribution and structure of the coated scaffolds was investigated. The FA dip-coating slip with ~1.0 mPa.s viscosity did not lead to a continuous film along the strut network and the macropores remained uncoated. The coating with FA slips having 2.2 mPa.s viscosity was uniformly distributed along the strut surfaces and inside the macropores forming a thin FA adhered layer, which did not significantly alter the porosity, the pore size and the strut thickness distribution of
the original ZrO\textsubscript{2} scaffolds structure. On the contrary, the greater amount of FA introduced into the scaffolds after coating with slips of 5.0 mPa.s viscosity resulted in thicker FA layers which strongly reduced the pore size and increased the strut thickness of the original ZrO\textsubscript{2} scaffolds. The porous structure obtained after coating with slips of 2.2 mPa.s viscosity, with a low reduction of the porosity and pore size by the FA layer and a continuous film distributed along the strut surfaces, is expected to be optimum for tissue ingrowth and bone formation.

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