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Synthesis of biocompatible surfaces by different techniques

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ABSTRACT

In the present work, a sol-gel method of HA synthesis has been developed using different calcium and phosphorous precursors. In order to make a porous scaffold, HA aerogel formation and HA liofilization processes have been studied. The liofilized HA and the HA aerogels have been characterized by SEM-EDX and X-Ray difraction. Finally, so as to improve the biocompatibility and the bioadhesion of the HA scaffolds, different plasma polymerization techniques have been studied. Acrylic acid has been polymerized using plasma polymerization with a radiofrequency source. Different radiofrequency source powers and reactor designs have been tryed. The kinetics of the thin film growth have been studied and the optimal polymerization conditions have been established. Polymeric layers have been characterized by IR and XPS.

INTRODUCTION

Regeneration or replacement of damaged tissues remains one of the most important challenges in both medicine and bioengineering. Specifically, methods of bone/cartilage reconstruction in orthopaedic surgery are not completely successful yet. In addition, they are among the most costly therapies avaiable. Materials science can help in finding new suitable materials for bone/cartilage regeneration, and this is why it represents a major area of study in tissue engineering [1].

Green [2] postulated correctly that the advent of new biocompatible materials might enable cells to be seeded onto a synthetic scaffolding and implanted into animals to generate new functional tissue. Scaffold or three-dimansional construct initially provides mechanical stability as well as cell anchorage sites and structural cues. Furthermore, the scaffold must also provide an appropriate environment to enhance cells reorganization and the correct extracellular matrix generation by these cells [3]. Several scaffold materials have been investigated for tissue engineering bone and cartilage, and several reviews have been published on the general properties and design features of biodegradable and bioresorbable polymers and scaffolds [4-9]. Natural materials are advantageous in that they contain information that facilitates cell adhesion or maintenance of differenciated function. Synthetic polymers, in the other hand, allow precise control over molecular weight degradation time, hydrofobicity and other attributes [1]. The advantages of both have been tryed to combine in different strategies [7].

This work is a part of a global project that wants to create a biomaterials program for bone and cartilage regeneration. The main goal is to develop biocompatible and bioresorbable scaffolds that restore, maintain or improve bone and cartilage function. It is important that these scaffolds give mechanical resistance in the reconstructed area, but also they must be osteoconductives in order to promote the area reconstruction by a new bone or cartilage.

Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, is a crystalline form of calcium phosphate, very similar to the mineral component of bones. It has been studied for bone and cartilage regeneration because of its biocompatible, bioactive and osteoconductive properties, which make it a good biomaterial for bone/cartilage repair [10-12]. Thus, HA has been chosen in this work and it has been synthesized by an optimized sol-gel method. Sol-gel synthesis of HA ceramics has recently attracted much attention [13-14]. The sol-gel method is advantagous for different reasons. The most important are that, temperatures required for all stages are low, different shapes can be given easily to the formed gel and by controlling the aging and drying conditions, further pore size and mechanical strength control may be achieved [15]. Moreover and in order to improve the osteoconductivity of the obtained HA, two different drying methods for the HA precursor gels are proved: liofilization and aerogel production. In the other hand, polymers are widely used in bone/cartilage implants, both, as polymeric materials themselves and as a polymeric surface on a different substrate. Concerning the later, and in order to improve the biocompatibility of the material, surface modification techniques appear to be quite interesting for this subject. Thus, surface modification techniques become a good choice to improve the biocompatibility of the material. Specially, plasma surface modification has been used as a promising technique for this purpose [16-17]. Acrylic acid deposition by plasma polymerization with a radiofrequency source has been studied in this work. The conditions of power, pressure and reactor design are very important because they affect the polymerization process and, thus, the final polymeric layer properties obtained. The next step will be to modify HA scaffolds surface in order to attach interesting biomolecules, such as growth factors, or to improve cell adhesion, biocompatibility, and bioactivity.

Therefore, the work will focus on the production of HA scaffolds by a sol-gel method using different drying processes, and on the study of the plasma polymerization technique to design modified HA scaffolds for bone and cartilage regeneration.. These modified HA scaffolds are promising substitutes for bone/cartilage repair because of its ability to give an appropriate environment for cell proliferation and, therefore, for the growth or replacement of damaged tissues.

EXPERIMENTAL DETAILS

Synthesis of hydroxyapatite (HA) by a sol-gel method

Triethylphosphite or triethylphosphate(Aldrich) were chosen as phosphorous precursors Calcium Nitrate and Calcium acetate (Aldrich) were used as calcium precursors.

The reaction was carried out at room temperature in a vessel equipped with a cooling system to avoid losses of the volatile phosphorous precursors during the sol formation. First the phosphorous precursor was diluted in anhydrous ethanol and then, a small amount of distilled water was added for hydrolysis, keeping a molar ratio of water to the phosphorous precursor at 3.

A stoichiometric amount (to maintain Ca/P= 1,67) of the calcium precursor solution (3 M solution in anhidrous etanol) was added dropwise into the hydrolyzed phosphorous sol. The pH of the sol was maintained around 2,8 Vigorous stirring was continued for an additional 25min after the titration. A clear solution was obtained and aged at room temperature for 24h before drying. The solvents were then driven off at 60° C until a gel was obtained. Besides, an aqueous-based process was employed with distilled water as the only diluting medium [13].

The gel compositions were analyzed by EDX (Oxford Scientific) and TGA (Mettler- Toledo TGA 50.

The gel was subjected to different drying processes: calcination, liofilization and aerogel formation.

In the calcination process the gel was subjected to different temperatures, from 300 to 900 °C, for 2, 4 or 6h intervals. The presence of HA phase was evaluated using an X-ray diffractometer (Bruker D-5005).

Liofilization drying process

This process was performed in standard liofilization equipment. The gels for liofilization must be in aqueous medium. The rate of cooling will influence the structure of the frozen matrix. Thus, different cooling systems were used to freeze the samples: with liquid N_2 (-173 °C), at –78 °C with CO_2/acetone and at –20°C in the freezer.

Aerogel production

Aerogel production was carried out in a reactor, which has the following characteristics: maximum pressure of 500 bar, maximum temperature of 400°C, heating capability of 14500W, maximum CO_2 flow of 40Kg/h, maximum solvent flow of 1,8 L/h and a computer aided system. The supercritical cycle was composed of 4 stages: pressure increase until 150 bar followed by temperature increase until 250 °C, where the system reaches the supercritical conditions. Then, pressure decrease until atmospheric pressure and after that, temperature decrease until room temperature. The HA aerogels were characterized by X-ray diffraction and SEM-EDX.

Plasma polymerization

Acid acrylic was polymerized onto glass and HA substrates through plasma polymerization technique at low pressure, with a radiofrequency source of 13,56 MHz. Different reactor designs were employed and different monomer pressures were tried. One of the apparatus used for the plasma treatment consists of a stainless steel discharge vessel (diameter 26 cm, length 24 cm) with internal electrodes which are connected to a radio frequency (RF) ACG-3XL generator at 13,56 mHz from ENI (Italy). The gases are supplied via a standard manifold with gas fluxes adjusted with needle valves. A two stages mechanical pump(D8B from Leybold (UK)) evacuates the vessel

The second one is a stainless steel vessel (diameter 60 cm, length 45 cm) equipped with three different trays (polymerization positions). These are connected to a radio frequency (RF) at 13,56 MHz . The gases are supplied via a standard manifold on the rear part of the vessel. Also a two stages mechanical pump evacuates the vessel.

The pressure were varied between 0,1-0,8 mbar and the RF power between 15-40 W .

The kinetics of the thin film growth was also studied. Layers were also characterized by IR (FTIR Nicolet Manga 560) and XPS (PH 5500 Perkin Elmer).

DISCUSSION

Synthesis of HA by a sol-gel method

Researchers employ a number of combinations between calcium and phosphorous precursors. Besides the difference in chemical activity of the precursors, such as, hydrolysis, polycondensation,etc, the temperature that is required to form the apatitic structure depends largely on the chemical nature of the precursors [13]. In the present work, experimental conditions of HA gel synthesis have been optimized, trying different calcium and phosphorous precursors and different solvatation medias, as it is explained above. The gel ageing time is also important for the solution system to stabilize, and therefore, to obtain later the HA phase.

Hydrolisis activity of triethylphosphate is relatively poor compared with the triethylphosphite one, and a higher solution temperature together with a prolonged time period is

needed to form the HA phase [15]. That could explain why results obtained in this work with triethylphosphate were not satisfactory. In the other hand, calcium acetate was found to be less soluble in the media used than calcium nitrate, and that affected to correct sol formation.

Before doing the thermal treatment to the gels in order to obtain the HA phase, they were characterized by TGA. Gel TGAs all show a first weight loss from RT to 250 °C due to solvent evaporation. Then, no weight loss was observed until 400 °C, if calcium acetate was used as calcium precursor or until 550 °C, if calcium nitrate was used. These temperatures correspond to the decomposition temperatures of calcium precursors, as TGAs of them have confirmed.

The thermal treatment consisted of heating gels at different temperatures for different periods of time in order to find the best formation conditions of HA phase. HA phase often appears accompanied of other phases that can be different depending on the precursors used, the thermal treatment temperature and the time interval, as table I shows. The more the thermal treatment time is increased, the more HA formed at one fixed temperature becomes crystalline.

The different phase transformations of calcium precursors were observed in XRDs as a temperature function. For instance, calcium acetate was transformed to calcium carbonate at 450 °C, at 700 °C carbonate was transformed to hydroxide and finally calcium oxide was obtained. TGAs of calcium precursors and gels also confirmed it. It can be concluded that HA phase formation depend on the decomposition temperature of the calcium precursor used. Thus, the degree of carbonatation can be determined by TGA analysis. This parameter is directly related with the calcium precursor used. The maximum carbonatation level (~25%) is achieved using calcium acetate.

			Thermal treatment temperatures /°C						
	Calcium precursor	**Thermal treatment time /h**	**300**	**400**	**500**	**600**	**700**	**800**	**900**
Triethylphosphite	Nitrate	2	-	HA, $CaCO_3$	HA, $CaCO_3$	HA, $CaCO_3$	HA, $CaCO_3$	HA, W, $CaCO_3$	
	Acetate	2	Amorfous			W, $Ca_2P_2O_9$	HA, W	HA, W, $Ca(OH)_2$, CaO	
	Acetate	4	HA*	-	-	HA	HA, W	HA, W	HA, W, CaO
	Acetate	6	HA* W	-	-	-	HA, W	HA, W	HA, W, CaO

Table I. HA*: poor crystalline hydroxyapatite, W: whitlockite

Calcium nitrate and triethylphosphite in aqueous media have shown better feasibility to form the sol and in the same way the gel obtained from these precursors formed crystalline HA at lower temperatures as table I shows. Although it seems not necessary to obtain very pure and crystalline HA for bone and cartilage regeneration, (mineral component of bone is formed by myriad tiny, imperfect crystals), the use of calcium nitrate as precursor allows a better control on HA cristallinity. In this sense the HA formed could be easier tailored using this precursor.

Liofilization drying process and aerogel production

As we stated before, the precursors chose to make these scaffolds were calcium nitrate and triethylphosphite.

Liofilization process was used to dry hydrogels in order to obtain a porous structure. If the water freezes quickly (with liquid N_2), it could be expected that final product has a finer pore structure. If freezing is slower (at –20 ºC in the freezer), the resultant product may have courser pore structure. But sometimes it becomes convinient to be able to control the pore size more exactly. Then, aerogel production appears to be a quite interesting technique. Using both techniques, HA scaffolds were obtained and characterized by XRD and EDX-SEM.

The morphology of the samples, studied by SEM, shows, as it was expected, a higher open porous structre for the aerogels, with very small pores. This higher porosity of the aerogels could be important to provide osteoconductivity to the scaffold.

It is important to pointed out that, whereas liofilized structures need a post-thermal treatment to form the apatitic structure, in the case of aerogels (figure 2), HA accompanied of other phases was formed during the aerogel production process, as XRD of aerogels have proved (figure 1).

. **Plasma polymerization**

Different RF source powers monomer pressures, polymerization times and reactors designs were studied and optimal conditions were established for acrylic acid plasma polymerization onto glass and HA substrates (Power 9W, time 5 h, monomer pressure 0,01 mbar). This yields uniform layers of 50 nm thickness with good adhesion both to HA aerogels and glass plates. The more the RF power was increased, the more the polymerization occurred and layers obtained were not uniform, regarding the thickness uniformity on glass plates. Besides, the visual aspect corroborates the lack of uniformity. Layers deposited using a lower RF source power and a higher polymerization time, were uniform and had a high degree of acidic functional groups in its surface as IR analysis show (figure 3).

CONCLUSIONS

Promising results to design modified HA scaffolds are obtained.

Calcium nitrate works better than calcium acetate as calcium precursor for HA formation. The reactive preparation is easier to handle and the HA crystalinity can be better tailored.

The aerogel processing technique seems to be a very interesting technique to obtain osteoconductive HA scaffolds in one step with pore size control.

Plasma polymerization of acrylic acid at low RF source power leads to uniform functionalized layers with good adhesion to HA aerogels.

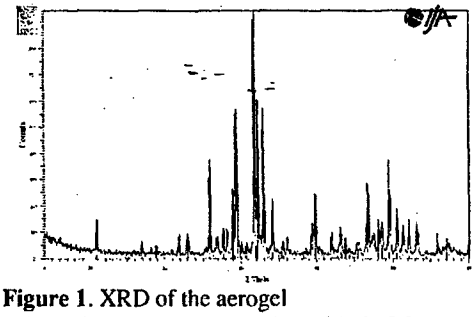

Figure 1. XRD of the aerogel
(HA is the main phase together with $CaCO_3$)

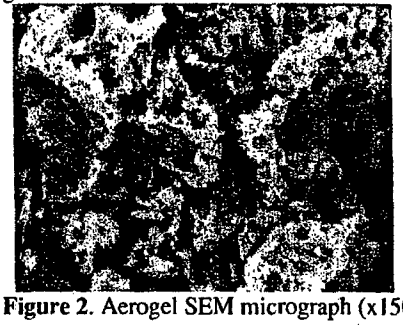

Figure 2. Aerogel SEM micrograph (x1500)

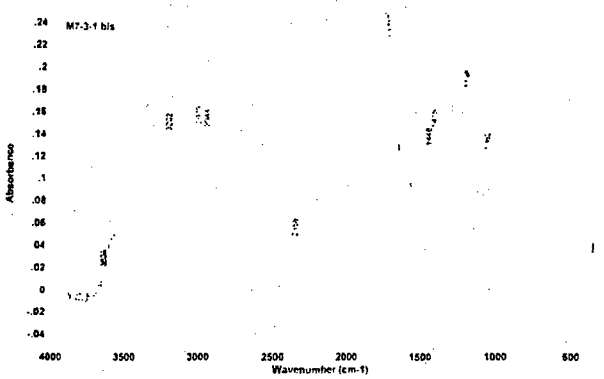

Figure 3.

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