Manufacture and morphological characterization of osteoconductive porous scaffolds

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Abstract

This paper discusses the manufacturing, chemical, and physical characterization of a three-dimensional porous scaffold developed for the repair of skeletal defects. Autografts from patient donor sites are the current gold standard. However donor sites morbidity and a limited material supply restrict this treatment option. This is the main reason why the presence of synthetic biomaterials has grown significantly over the past two decades with applications in orthopedics. Bone consists of cortical and spongy regions both composed of $Ca_{8,3}$ $(PO_4)_{4,3}$ $(HPO_4,$ $CO_3)_{1,7}$ $(OH,CO_3)_{0,3}$ mineral phase. Spongy bone is a highly spongy tissue (55-70% interconnected porosity) that allows the in-growth of the soft tissues and organic cells into the bone matrix. Chemically sub-micron and high phase-purity hydroxyapatite powders (HA: $Ca_{10}(PO_4)_6(OH)_2$) were mixed, in a centrifuge ball mill, together with ethanol (EtOH) and hydroxy-propyl-methyl-cellulose (HPMC) powders in appropriate amounts. The mixtures produced in this way were slowly dried in an oven. Dried powders were formed under pressure in cylindrical cakes and heated in an air atmosphere to the optimum sintering temperature. The raw materials and the scaffolds obtained by this technique were characterized by: XRD, laser granulometer, DSC-TGA analysis, FT-IR spectroscopy, SEM micrographs, and porosity measurements. This method produces porous HA scaffolds very similar to spongy bone from a chemical, physical and morphological point of view. The key parameters to the performance of the material for osteomimetic purposes will be analyzed and their effects studied also with reference to desirable modeling efforts.

1 Introduction

Bone is used annually in more than 450,000 graft procedures in the United States and in 2.2 million procedures worldwide to repair bone defects caused by either trauma or tumor resection and to achieve spinal arthrodesis [1]. Autografts obtained from patient donor sites are the current gold standard for skeletal defect repair. It provides all 3 interdependent elements necessary to maximize bone-forming ability: scaffolding for osteoconduction (bone mineral and collagen), growth factors for osteoinduction (noncollagenous bone matrix proteins), and progenitor cells for osteogenesis [2]. However, patient donor site morbidity and a limited material supply restrict the utility of this treatment option. When reporting results of clinical problems, investigators usually focus on the treatment outcome of the principal clinical problem and not on donor site morbidity from harvesting bone graft. Previous authors have reported, on the morbidity of iliac crest bone graft harvest, overall complication rates up to 49% [3]. A more detailed study by Younger and Chapman [4] found that the overall major complication rate was 8.6%. Major complications included infection (2.5%), prolonged wound drainage (0.8%), large hematomas (3.3%), reoperation (3.8%), pain over six months (2.5%), sensory loss (1.2%), and unsightly scars. Minor complications (20.6%) included superficial infection, minor wound problems, temporary sensory loss, and mild or resolving pain. Allograft bone obtained from tissue donors and synthetic bone cements are also suitable defect treatments. Allograft is the preferred substitute when autografting is not a realistic option. However, allograft tissue is expensive, yields more variable clinical results than autograft, and has known risks of bacterial contamination, viral transmission, and immunogenicity [5]. To lessen the potential risks to the recipient, allograft bone is intensively treated prior to preservation for storage; these processes contribute to increased costs and diminished mechanical and biologic properties. Bone cements, such as those based on poly(methyl methacrylate) (PMMA) can fill defects of variable shape and size. However, tribological effects, interfacial failure and stress shielding can lead to long-term complications and eventual revision [6]. Other synthetic materials, such as osteoconductive ceramic based composites, can be used as bone scaffolds Osteoconduction is a three-dimensional process observed when porous structures are implanted into or adjacent to bone tissue. As a matter of fact it refers to the ability of some materials to serve as a scaffold on which bone cells can attach, migrate (meaning move or "crawl"), grow and divide. In this way, the bone healing response is "conducted" through the graft site. Osteogenic cells generally work much better when they have a matrix or scaffold to attach to. Osteoconductive materials have the potential to provide suitable alternatives to autograft and allograft bone while also providing the capability to be custom manufactured with respect to the patient and target application. A variety of synthetic bone grafts have been tried, including ceramics, collagen, noncollagenous proteins, bioactive glasses, and biodegradable polymers; they are used in approximately 10% of bone graft procedures performed worldwide [1]. No single material, however, can satisfy all the goals required for manufacturing

optimal scaffolding in terms of mechanical properties, controlled degradation and osteoconductivity. Capillaries, perivascular tissues, and osteoprogenitor cells migrate into porous spaces and incorporate the porous structure with newly formed bone. The observed process is characterized by an initial in-growth of fibrovascular tissue that invades the porous structure followed by the later development of new bone applied directly within it [7]. One possible solution is to employ composites of biodegradable polymers and calcium phosphates ceramics as a method to exploit the advantages of each. Attention has been focused on the realization of a porous bioceramic scaffold, that while allowing loading transmission through the bulk material improves interface cohesion between natural bone and synthetic scaffold. Considering that the medium size of a human osteon is 223 μm, the optimal scaffold pore size, to support new bone growing, seems to be about 200 μm [8] with total porosity values between 35-55% [9]. Porosity is an essential requisite because it allows a quick colonization and better vascularization limiting the risks of ischemia injuries and penetrating cells necrosis. In this paper a newly developed process for fabricating ceramic scaffolds is described. The key parameters to the performance of the material for an osteomimetic purpose has been analyzed and their effect studied also recurring to modeling efforts. This suggests avenues for improvement in the formulation, in terms of the amount of polymeric agent, that affect the final porous structure and consequently the bone-implant cohesion. *In vitro* tests of these scaffolds are underway and we think that this manufacturing technique has a promising potential for future applications.

2 Materials and methods

Since DeJong first observed the similarity between powder X-ray diffraction pattern of the *in vivo* mineral and the HA in 1926, calcium phosphate ceramics have received attention as bone substitute material [10]. As a matter of fact HA has a strong resemblance with the mineral phase of bone: $Ca_{8,3}$ $(PO_4)_{4,3}$ $(HPO_4, CO_3)_{1,7}$ $(OH,CO_3)_{0,3}$ [11]. In order to develop a synthetic bone scaffold, an hydroxyapatite powder, HA - $Ca_{10}(PO_4)_6(OH)_2$ (Riedel-de-Haen), was selected and chemically analyzed, resulting in the spectrum shown in figure 1. The location and magnitude of the peaks in the spectrum attest to the purity of the material. This was mechanically mixed with HPMC (hydroxy-propyl-methyl cellulose) (Acros Organics). The mixture was prepared in different percentages, and the composite subsequently sintered. During the thermal treatment the polymeric binder burns out leaving in the ceramic matrix a porous network that is very similar to natural spongy bone tissue (the latter shown in figure 2) in the sense that volume and distribution of the porosity are similar.

HA and HPMC were mixed in a ball-mill (Fritsch Pulverisette). Two different sets of samples were realized. The samples labeled as D (dry mix) derive from a 30 minutes dry ball-milling of the starting raw materials. The ones labeled as W (wet mix) derive from a 15 minutes wet ball-milling. Wet means that the starting powders were milled together with ethanol (C_2H_5OH). After mixing the composite powders were pressed into a tablet shape, shown in figure 2, at 200

Mpa. The tablets thus obtained underwent sintering treatment in an air oven at 1100°C (dwell time 3 hours) followed by natural air cooling to room temperature, and experienced a reduction in volume, as shown in figure 2. The raw materials and the sintered tablets underwent chemical and physical characterization in order to evaluate the morphology of the obtained compounds and to compare them to the bone tissue.

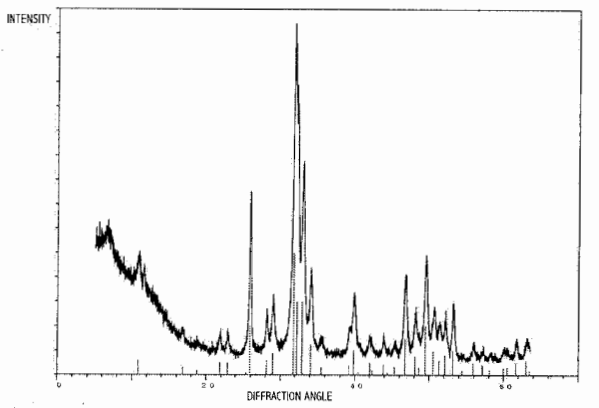


Figure 1: Hydroxyapatite (Riedel-de-Haen) XRD spectrum

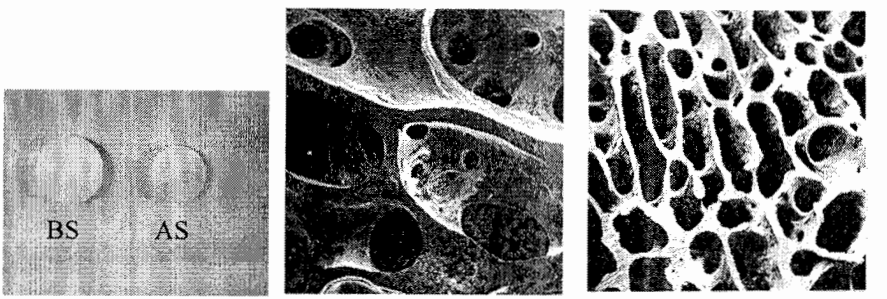
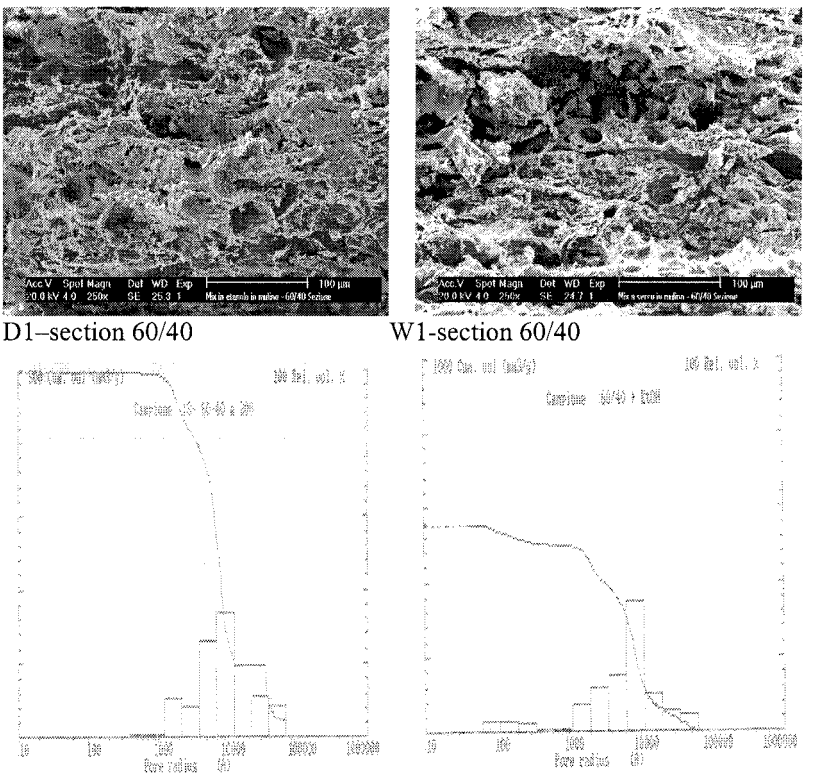


Figure 2: Powder table before (BS) and after (AS) sintering. Hydroxyl group released from HA causes a volume reduction of the sample after the thermal treatment (left); SEM micrographs of cortical human bone tissue (center) and spongy bone tissue (right).

3 Characterization

Scanning electron microscopy was used in order to visualize the pore morphology distribution in the bioceramic samples. Representative microstructures are shown in figure 3.

For porosity measurements, the sintered samples were analyzed in a mercury intrusion porosimeter. The results are summarized in Table 1, while the porosity distributions are shown together with the corresponding morphology in figures 3 and 4. Total porosity values over the range 20-75% were obtained, consistent with total porosity value of bone tissue. High porosity was expected to better satisfy the cell penetration requirements for osteoconductive purposes. Porosity and pore morphology of these samples were easily controlled by essentially changing the polymer concentration and ceramic content.



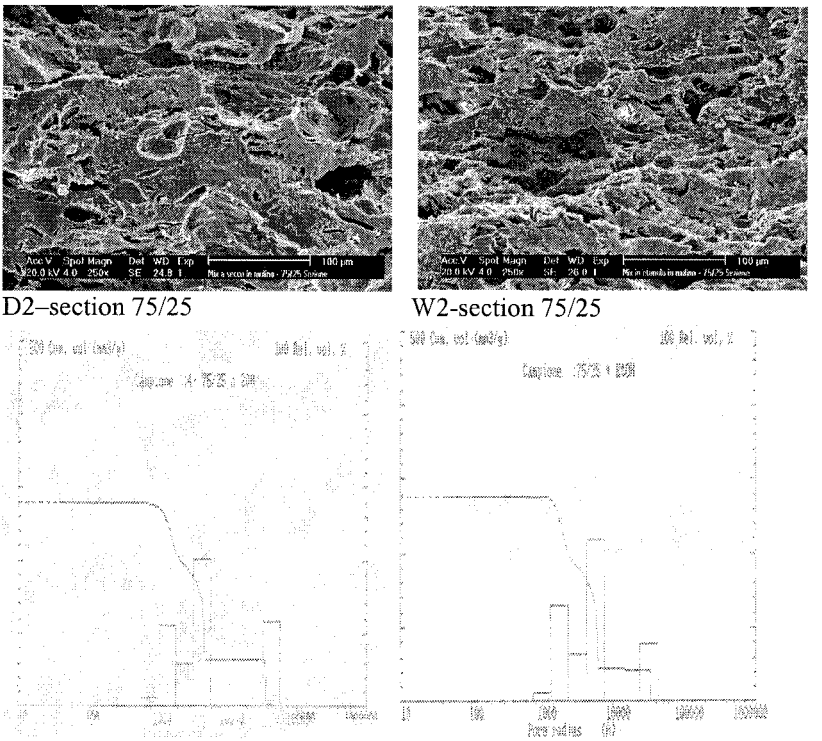
D1–section 60/40 W1-section 60/40

Figure 3: SEM micrographs of the microstructure resulting from a 60/40 HA/HPMC blends, dry and wet, with corresponding porosimetry plots.

In both cases, the structure of the ethanol-mixed scaffold displays more homogeneously dispersed pores. In addition, the distribution of pore sizes appears narrower, and the average pore size larger. Because there is no inherent disadvantage to using the wet preparation technique, it appears that this is preferable over the dry technique.

Unfortunately, although the pore structure resembles that found in natural tissue fairly closely, in all cases the average pore size is smaller than the ideal one found in biological bone tissue, with possible adverse consequences on the ability of osteoprogenitor cells to migrate freely through the scaffold. This

problem could be resolved by using a polymer dispersion with larger average size, and perhaps by achieving mixing by means other than ball.milling. These avenues are currently under investigation.



D2–section 75/25 W2-section 75/25

Figure 4: SEM micrographs of the microstructure resulting from a 72/75 HA/HPMC blends, dry and wet, with corresponding porosimetry plots.

Table 1: Total porosity values of the samples.

Sample	Total porosity value (%)
HA/HPMC 100/0 D	25%
HA/HPMC 75/25 D	64%
HA/HPMC 60/40 D	75%
HA/HPMC 100/0 W	20%
HA/HPMC 75/25 W	54%
HA/HPMC 60/40 W	74%

FT-IR analysis was performed on the W and D samples. The results, shown in figures 4 and 5, indicate that mixing in ethanol did not change the spectrum of the composite – in other words, no chemical reactions are initiated by either the presence of the polymer or the ethanol.

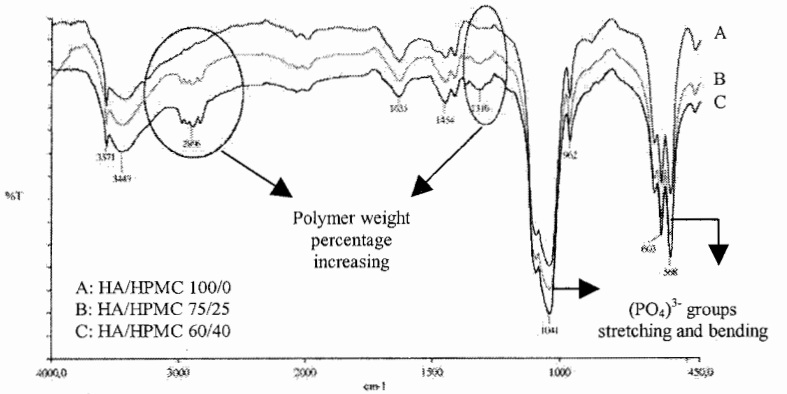


Figure 5: FT-IR spectra: comparison between three different samples manufactured with different polymer binder percentage.

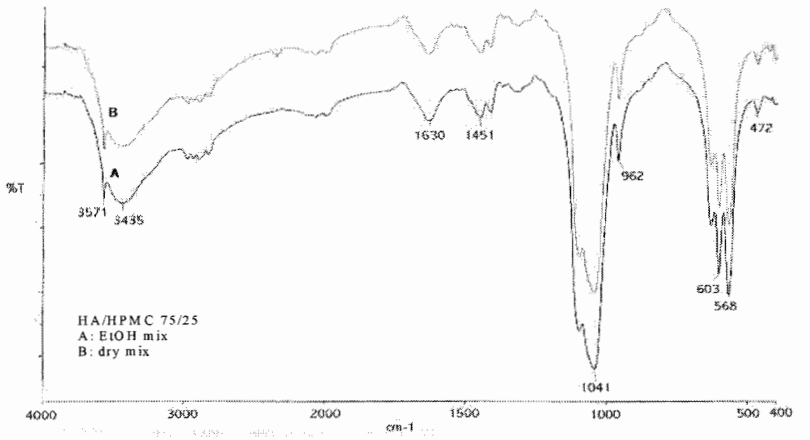


Figure 6: FT-IR spectra: comparison between samples, containing 60/40 ceramic/polymer proportions, manufactured under dry and wet mix conditions. The location of the peaks indicates that no chemical reaction is induced by the presence of the ethanol. Similar results are obtained for the 75-/25 composite.

Porosity seems to be a key parameter to the performance of a material for an osteomimetic purpose. Applying traditional techniques to characterize, from a chemical and physical point of view, this kind of bioceramic materials we have established that, while the overall porosity and pore structure are similar to those of natural bone tissue, the pore size is not. Given the fact that traditional manufacturing methods provide limited control over scaffold architecture and

pore geometry, it is possible to suggest avenues for improvement in the formulation, recurring to modeling efforts, in terms of the amount of polymeric agent, that affect the final porous structure and consequently the bone-implant cohesion.

4 Modeling considerations

In this section, aspects of the above materials that could best be addressed by modelling are considered. Currently, scaffolds are not used for bone replacements with structural function, because their mechanical properties are inadequate to support loads encountered in practice, while the time required for the regrowth of bone tissue (approximately eight months), which would provide adequate strength to the implant, are too long to keep a patient immobilized. Thus, presently bone scaffolds are not a feasible alternative to conventional metal bone replacements except in implants not subjected to significant loading, such as small areas in the skull. However, the situation might change if the mechanical properties of the materials could be improved substantially.

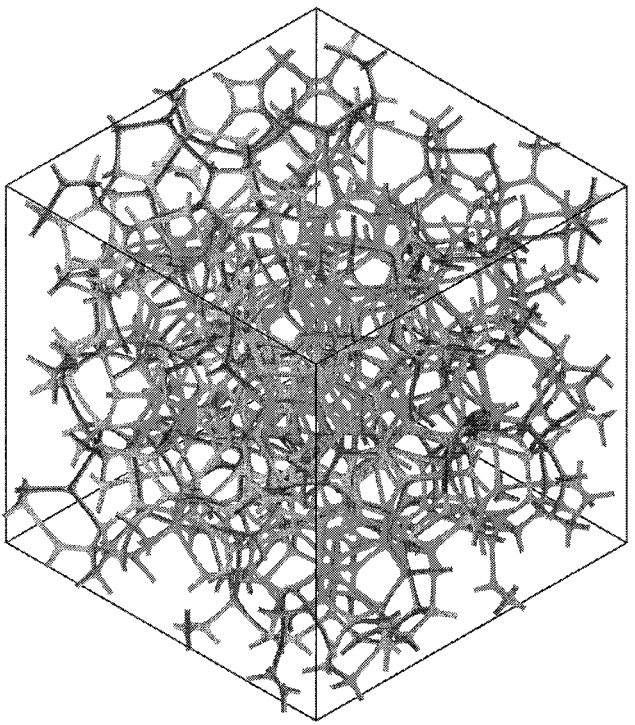


Figure 7: Repeat cell model of a porous scaffold. The porosity is much larger than would be utilized in practice.

Clearly, the ideal microstructure of the bone implant would mimic that found in biological bone tissue, shown in Fig. 2. This could be achieved by utilizing HPMC or other biocompatible powders where the grain size distribution is similar to the pore size distribution of bone, with HA powder of much smaller dimensions. The HA powder would fill the interstices between the polymer grains, forming a scaffold of the desired structure upon polymer burnout. Because of the high cost of biocompatible raw materials, experimental optimisation of the scaffold structure is not practical.

The numerical modelling would then begin from the simulation of the blending and sintering process. A distribution of spherical particles, representing the biocompatible polymer, is produced with the required volume fraction (similar to the porosity of the bone being replaced). The volume between the particles is the scaffold. A surface discretization of the scaffold, obtained with a monomodal distribution of spheres, is shown in Fig. 6.

The parameters than can be controlled in the manufacture of the scaffold are the volume fraction of polymer, and its polydispersity. Optimization can be performed with respect to parameters of interest, for example the maximum tensile loading or the maximum principal tensile stress. In addition, a sensitivity analysis can be performed with respect to possible defects in the structure, such as the effect of a broken or missing strut.

5 Conclusions

A promising procedure for producing a porous scaffold for the repair of skeletal defects has been developed. Characterization by various experimental techniques indicates many similarities with human skeletal tissue, including porosity and chemical composition. However, some improvements can still be made, both morphological and in terms of mechanical properties. In fact, the two are linked, and are best optimised by simulation, prior to their actual fabrication, owing to the high cost of the raw materials. The parameters that can be controlled, and hence optimised, are the porosity and the polydispersity of the constituent powders, polymer and HA. A new technique for generating the surface discretization of a structure that is morphologically very similar to bone tissue was developed. Software capable of analysing the stresses in the structure due to arbitrary loading is currently being developed.

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