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Foam-Based Bionanocomposite Scaffold for Bone Tissue Engineering

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Abstract. The repair of large bone defects is a major clinical problem for which tissue engineering (association of a biomaterial and cells) constitutes a valuable alternative. In this domain, the architecture and the mechanical properties of the 3D scaffold aimed to support cells is of key importance to succeed in bone reconstruction.
In this study, we aim to design and evaluate a bionanocomposite foam-based scaffold, exhibiting all the desired biofunctional attributes of biocompatibility, bioactivity, osteoconduction/induction, combined with potential release properties. To perform this, 2 components have been associated: (i) a biopolymer, pectin, incorporating (ii) calcium phosphate nanoparticules to provide bone apatite nucleation sites, mechanical reinforcement, and to play the role of potential drug reservoir. The goal of this study was to determine the feasibility of obtention of such bionanocomposite by foam-templating, and to study the influence of mineral particules ratio on pectin foam and final scaffold 3D architecture and properties.

Introduction

Damage to oral and maxillofacial tissues (due to congenital deformities, acute trauma, chronic nonunion, or resection of pathology), even when minimal, usually leads to noticeable deformities [1]. Therefore, the repair of large segmental bone defects of the jaw, palate or orbital floor remains a major clinical problem. Autograft, often regarded as the « gold standard » for bone replacement in the craniomaxillofacial region, has indeed several drawbacks: it implies a secondary surgical site which conveys additional pain for the patient and increases the morbidity rate and infection potentialities [2]. To remedy these shortcomings, tissue engineering, and more particularly the association of progenitor or stem cells with a biomaterial, generating a so-called hybrid material, constitutes a valuable alternative [3,4]. To fulfill large bone defects requirements, the ideal biomaterial should combine interconnected porosity, sufficient mechanical strength according to the implantation site, and angiogenic factors release ability.
The goal of this work is to evaluate the feasibility of using pectin and nanocrystalline apatite to obtain a bionanocomposite, tailorable for maxillofacial reconstruction, exhibiting all the desired biofunctional attributes of biocompatibility, bioactivity, osteoconduction/induction, and potential release ability.
Foam templating has recently demonstrated its interest for the elaboration of biopolymer 3D scaffolds that can be seeded with mesenchymal stem cells for cell therapy [5]. Although promising, this technique has never been tested in presence of mineral charges, for bone applications. Our aim is to associate, for the first time, nanostructured mineral phases to this type of scaffolds in order to

specifically adapt them to bone reconstruction problematics. To design this bionanocomposite, 2 components will be associated: (i) pectin (PEC) will insure the 3D architecture, interconnected porosity and elasticity of the material aimed to be cell seeded; (ii) nanocrystalline apatite particles distributed in the matrix will provide bone apatite nucleation sites and mechanical reinforcement. The rationale of choice of the biopolymer lies in its biocompatibility, biodegradability and recently demonstrated interest for bone reconstruction [6-8]. However, foam templating has never been applied to this biopolymer and the biocompatibility of the resulting 3D-scaffold needs to be explored. The use of a copper-doped apatite will further permit to optimize its bioactivity (by combining antibacterial and boosted angiogenic effects) [9].
We present here preliminary studies of the feasibility of such bionanocomposite. The necessity of surfactants (to stabilize the mineral phase dispersion and optimize porosity) and the contribution of the mineral charge (to improve mechanical resistance) have been particularly studied to determine the optimal scaffold formulation.

Materials and Methods

Pectin 916, Low methoxy amidated pectin, was kindly supplied by CP Kelco (Novamatrix, Norway). Montanox and Pluronic surfactants were provided by Seppic (France) and BASF Corporation (France), respectively.

Biomimetic nanocrystalline apatite synthesis. Apatite was synthesized as previously described [10]. Optimal conditions of dispersion by a microfluidizer were determined in order to obtain a D[3,2] of 5 µm (data not shown).

Macroporous scaffolds elaboration. 0.9% (w/w) sodium bicarbonate and 1% (w/w) surfactant (Montanox 20 or Pluronic F108) were added to of 3 % (w/w) pectin solutions and stirred 30 min at 1800-2000 rpm until a stable foam was obtained. Various amounts of apatite (ranging from 0 to 0.8% w/w) were added to the solutions. Three-dimensional scaffolds were generated by cross-linking in an iso-osmotic buffer containing calcium ions (150 mMNaCl, 0.1 M $CaCl_2 \cdot 2H_2O$, 0.3% w/w acetic acid) and freeze-drying.

Foam stability evaluation. Foam stability over time was evaluated by measuring the foam volume in a graduated test tube at determined time intervals.

Scanning electron microscopy (SEM). SEM analyses of surfaces and cross-sections of dried 3D scaffolds were performed with a Leo 435 VP scanning electron microscope. Samples were mounted on an aluminum sample mount and sputter-coated with silver. The specimens were observed at a 10 kV accelerating voltage.

Mechanical properties evaluation. Young moduli and mechanical behavior of the scaffolds were followed by uniaxial compressive assays (TA-XT2 Texture Analyzer, Stable Microsystems, UK). The apparatus consisted of a mobile probe (314.16 mm^2) moving vertically up and down at a constant and predefined velocity (0.5 mms^{-1}). The force exerted by the probe on the scaffolds was recorded as a function of the displacement. Then, the force was converted into stress by reporting the force to the surface of force application and the displacement was converted to a strain percentage in comparison with the initial dimension. Young moduli were calculated from the stress–strain curves at 50% of strain and represent the relative stiffness of the scaffold at 50% strain. The Young modulus was expressed as follows from at least three independent observations: $E_{50\%} = [(F_{50\%}/S)/Strain] \times 1000$ kPa, where $F_{50\%}$ is the force registered at 50% strain (N) and S is the surface of the specimen (mm^2).

Results and Discussion

Stability of pectin foams prepared using various surfactants. Pectin foams were produced by mixing 3% (w/w) pectin solutions with bicarbonate and a surfactant, in presence of various amounts of nanocrystalline apatite. 2 surfactants were tested as foam and suspension stabilizers: Montanox 20 and Pluronic F108. The foams stability was studied after 30 min of mixing (Figure 1). Foams appeared stable for 30 min, showing that the 2 surfactants selected for this study are adapted to prepare foam-based bionanocomposite scaffolds.

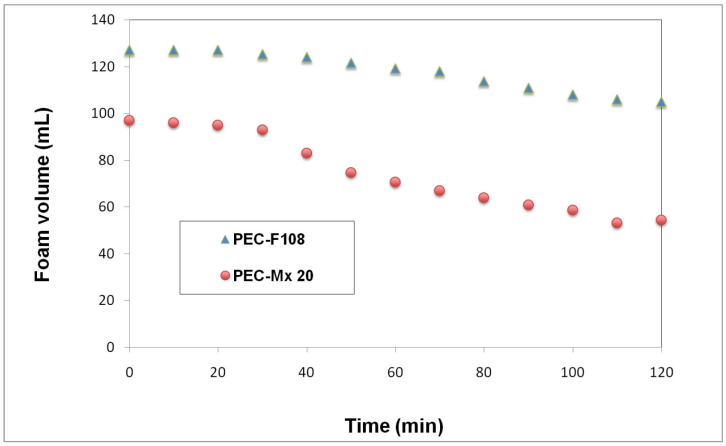

Figure 1: Foam stability over time

Morphology and porosity of foam based-scaffolds. After gelation and drying steps, different scaffolds were obtained in presence of various amounts of apatite and Montanox 20 or Pluronic F108 surfactants. Reference scaffolds without apatite were also prepared, according to the same procedure. Figure 2 shows SEM images of the cross-section of reference pectin scaffolds obtained in presence of Montanox 20 (PEC-Mx20- 0% AP: Fig. A), or Pluronic F108 (PEC-F108 - 0% AP :Fig. B), in comparison with scaffolds containing 0.5% of apatite (PEC-Mx20- 0.5% AP: Fig. C and PEC-F108- 0.5% AP: Fig. D). SEM micrographs reveal a highly porous and interconnected morphology of all freeze-dried scaffolds, with pore size ranging from 30 to 250 µm.

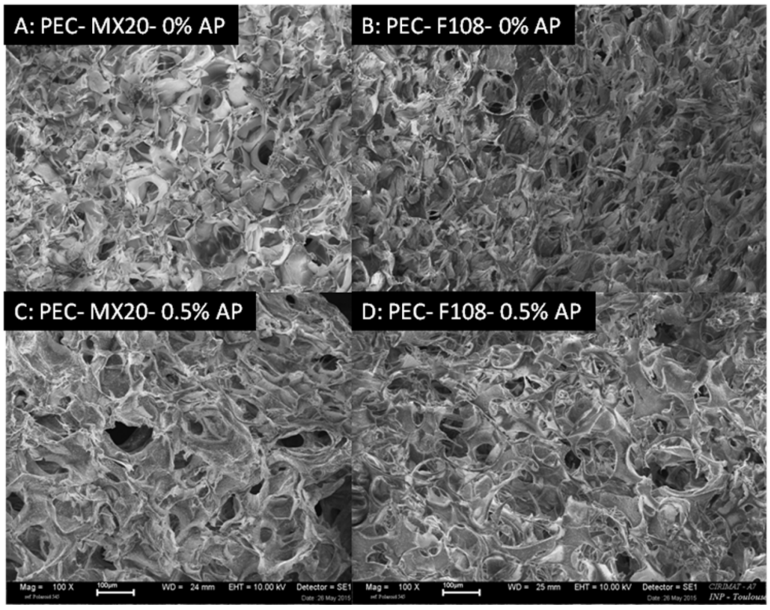

Figure 2: SEM photographs of bionanocomposite scaffolds in comparison to reference pectin scaffolds

Mechanical properties of 3D scaffolds under compression. Mechanical behaviors of rehydrated scaffolds were assessed by uniaxial compression and their Young moduli were determined at 50% of strain (Figure 3). The matrices prepared in absence of apatite presented lower mechanical

properties than the bionanocomposite scaffolds. Bionanocomposite scaffolds presented higher Young moduli, proportionally to their apatite content. A minimal content of 0.3% of apatite appeared necessary to obtain scaffolds with improved mechanical properties.

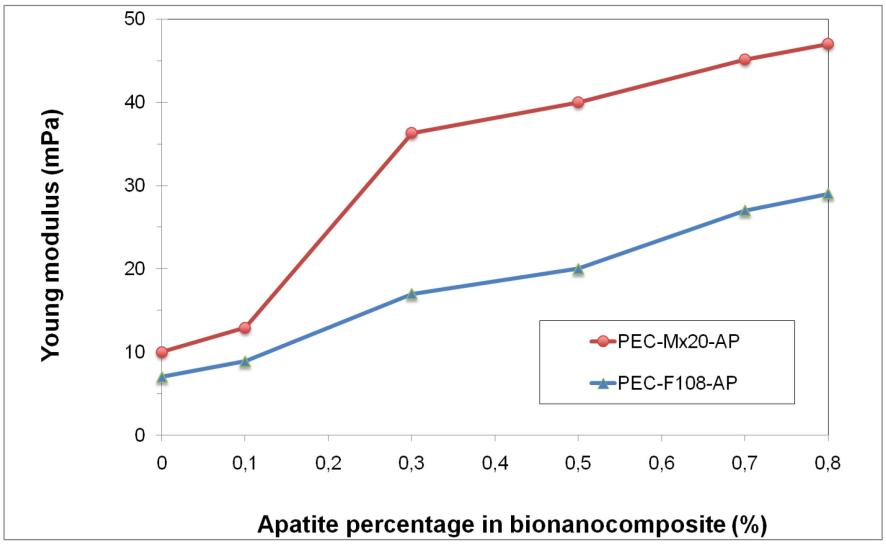

Figure 3: Young moduli of PEC-AP bionanocomposite scaffolds according to apatite content

Generating high porosity in implantable scaffolds is becoming a priority in tissue engineering and cell therapy. Indeed, an interconnected porosity has been reported to be indispensable to allow a good nutriment circulation, to promote entrapped cell migration and proliferation, and to improve the long-term efficacy of the implanted device by promoting tissue integration and neovascularization. A consensus has been established on the necessity to generate an interconnected porous structure with pore size ranging from 50 to 300 µm [11]. Among the different strategies described in the literature to generate porosity in biopolymer scaffolds, foaming techniques appear promising for tuning the porosity of the hydrogels [5]. However, this technique has never been tested with pectin to obtain scaffolds in association with nanocrystalline apatite. The present paper reports for the first time a feasibility study of bionanocomposite foam-based scaffolds for bone tissue engineering. To that aim, we have compared the influence of two surfactants and various amounts of apatite on foam stability and resulting scaffolds architecture and mechanical properties. We selected non-ionic, highly hydrophilic surfactants already used in biomedical applications. Surfactant addition to apatite-pectin solutions appears as an effective strategy to stabilize the suspension and foam and, consequently, to obtain a homogenous porous structure after cross-linking. The use of these surfactants permitted obtaining stable foams over 30 min, permitting to prepare foam-based scaffolds with a largely homogeneous porosity in all structures.

Scaffolds prepared using various surfactants and apatite amounts differed regarding their surface and cross-section porosity. Consequently, scaffold microarchitecture could be controlled by playing with these parameters. However, whatever the scaffolds formulation tested, surface porosity and pore interconnectivity appeared in a size range adapted for in-depth cell seeding. These results were confirmed by a preliminary *in vitro* biocompatibility study: 3D scaffolds PEC-MX20 and PEC-F108 were successfully in-depth seeded with human mesenchymal stem cells (MSCs) after centrifugation. Good cell viability results (higher than 70% after 14 days of culture, whatever the scaffold tested; data not shown) validate the biopolymer and surfactant choices.

Mechanical resistance is another critical parameter affecting the implantability of the final device and its *in vivo* fate. We show here that apatite addition can improve mechanical properties of the scaffolds without affecting interconnected porosity. Measurements of the Young moduli showed an increase of mechanical properties when apatite amounts were higher than 0.3%. The range of mechanical resistance obtained could be adapted for non-bearing implantation sites like palate and orbital floor.

Conclusion

Combining interconnected macroporosity and a mechanical resistance adapted to the implantation site is the technological challenge of all scaffold conception. In our study, we have shown the contribution of a mineral apatitic charge (to improve mechanical resistance) and the necessity of surfactants (to stabilize the mineral phase dispersion and optimize porosity) to obtain tailorable foam based-bionanocomposites. This preliminary study demonstrates the feasibility of macroporous pectin-apatite foam-based bionanocomposites that could be used as bioactive scaffolds (3D constructs) for mesenchymal stem cells implantation in large bone defects. Their functionalization and biological evaluation is the next step of this project.

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