New Degradable Load-Bearing Biomaterials Based on Reinforced Thermoplastic Starch Incorporating Blends

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This paper describes the processing, and correspondent performance of two starch-based blends (corn starch blended with ethylene vinyl alcohol or cellulose acetate) aimed at being used in temporary bone replacement and/or as tissue engineering scaffolds. The materials formulation, the processing routes, the controlled morphology development and the correspondent mechanical properties and in-vitro degradation behavior are discussed, in order to justify that these composites can be an effective alternative to the systems currently used in clinical practice. Furthermore, the effect of sterilization techniques and the possibility of growing Ca-P coatings on the material's surface (in order to promote osteointegration) is analyzed. Finally, the biological behavior of the composites is discussed on the basis of results obtained in in-vitro cell cultures and in-vivo goat implantation studies.

1. INTRODUCTION

There are several clinical and potential applications of biodegradable polymers in the biomedical field. For a particular material to be used in orthopedics it is particularly important that it can meet a highly demanding list of requirements. An ideal material to be used in temporary bone replacement should associate an adequate range of mechanical properties (matching those of bone), with a convenient degradation kinetics, a bone-bonding behavior and a biocompatible performance. To date there is not, yet, any material satisfactorily fulfilling all these requirements. Furthermore, there is a need for the development of polymeric architectures and morphologies that allow bone ingrowth, as the materials start to be degraded by the body fluids.

Recently, starch based materials have been proposed for this type of application (1–3). These materials present a unique range of properties that make them good candidates for temporary orthopedic applications.

This paper describes an oriented research work towards the development of alternative systems for use in bioresorbable load-bearing orthopedic applications. The studied polymers are based on starch blended with copolymers of ethylene and vinyl alcohol (SEVA-C) or with cellulose acetate (SCA). Both grades were reinforced with bioactive fillers. These fillers consist of bone-like ceramics, namely hydroxylapatite—a calcium phosphate hydroxide (HA), or calcium phosphate (Ca-P) based glasses and glass-ceramics. The composites were produced and molded by different melt processing techniques.

2. MATERIALS AND METHODS

Different types of starch based blends, Mater-Bi (from Novamont, Novara, Italy), were studied. These polymeric systems were obtained by blending native maize starch (70% amyloptectin and 30% amylose) with a synthetic copolymer. Two different blends were studied: (i) starch/poly (ethylene vinyl alcohol) (SEVA-C); and (ii) starch/cellulose acetate (SCA).

Both matrices were reinforced with bioactive fillers: i) hydroxylapatite (HA), Plasma Biotal, Tideswell, UK; and ii) calcium phosphate (Ca-P) based glasses and glass-ceramics in the SiO_2-$3CaO{\cdot}P_2O_5$-MgO system. These materials were prepared as described in previous works (4).

Hydroxylapatite was sintered at 1200°C for 12 hours, followed by crashing in a ball mill. Several granulometric distributions, ranging from an average particle size of 6.5 μm to 50 μm, were used both for HA and Ca-P glasses, to produce composites using filler amounts up to 30% by weight (10% step).

The composites were produced and molded by different melt processing techniques (twin screw extrusion, conventional and non-conventional injection molding), into several shapes and architectures, including structural foams. The effects of the injection molding conditions and specimen geometry on the achieved mechanical properties were studied on the basis of optimizing the materials semi-crystalline morphology (these polymers are known to be very sensitive to the shear stresses imposed to the melt during processing). So, the injection molding program included

the variation of the flow rate, the injection and holding pressures, the temperature profile along the barrel, and the gating geometry. A Klockner-Ferromatik Desma FM20, and a Krauss-Maffei KM-60/120A were used as basic molding equipments.

A non-conventional technology, shear controlled orientation in injection molding (Scorim), in a Demag D-150 NCIII-K fitted with a Scorim head, was used to further enhance the materials mechanical behavior, and induce anisotropy into the moldings with the aim of copying bone structure. This processing technique is based on the application of shear stress fields to the melt, during the holding and cooling stages of the injection molding, that tend to induce the development of a highly oriented morphology into the moldings (2, 5). For Scorim, among other parameters, the processing window included the variation of the maximum compactation pressures and the frequency of the hydraulic pistons (that shear the melt) oscillation.

Prior to injection molding the polymeric blends and HA (or Ca-P glasses) were compounded either in a rotating drum (RD) or by twin-screw extrusion (TSE) in a co-rotating Leitztritz equipment.

Structural foams (i.e. materials with a compact dense surface layer and a foamed porous core) were produced by conventional injection molding techniques using master-batches of blowing agents based on calcium and sodium containing carboxylic acids. These architectures, which copy the structure of cortical/cancellous bone, may be very useful as substrates for the tissue engineering of bone.

The samples were also coated, in a simulated body fluid (SBF) with a Ca-P bone-like layer, according to a previously described process (4), in order to induce on its surface a bioactive behavior. The bioactivity of the developed materials, uncoated or Ca-P coated, was studied in-vitro in a simulated body fluid (SBF). The effects of ethylene oxide (EtO) sterilization over the materials structure and mechanical performance were also studied.

The mechanical behavior of the injection molded parts was assessed in tensile, instrumented impact, creep and dynamic-mechanical analysis (DMA) tests. The molded specimens were conditioned and tensile tested under controlled environment (23°C and 55% HR) on a universal testing machine, Instron 4505, fitted with a resistive extensometer (gauge length, 10 mm). The mechanical behavior was characterized by means of the ultimate tensile strength (UTS), secant modulus of 1% strain ($E_{1\%}$) and the strain at break (ε_r). The fracture surfaces were observed by scanning electron microscopy (SEM).

The degradation of the polymers and the composites was studied in a simulated physiological solution (Hank's balanced salt solution, HBSS) with or without bovine serum additions (BS-10 and 30% v/v) at 37°C, for times up to 90 days. After several immersion periods the material's weight loss, water-uptake, structural changes and alterations on the mechanical properties were measured.

The biological behavior of the materials was assessed on *in-vitro* cell culture tests (using mouse lung L929 cells and PK-84 human skin fibroblasts) and on *in-vivo* goat implantation studies. Standard ISO MEM extraction cytotoxicity tests and MTT-quantitative assays were carried out. Samples were also implanted both intracortically and intramuscularly on Dutch milk goats for 6 and 12 weeks survival times.

3. RESULTS AND DISCUSSION

The material formulation, the selected processing routes, the type (and size) and amount of filler, and the control of the morphological development during processing have a direct effect on the correspondent mechanical properties and in-vitro degradation behavior. The integrated optimization of all these variables testifies that these composites can be an effective alternative to the biodegradable systems currently used in clinical practice. In general, the higher the orientation and the flow rates within the moldings, the better the mechanical properties and the slower the degradation rates.

The use of non-conventional processing techniques, such as Scorim associated to a proper compounding by TSE, allows for the elaboration of moldings with controlled anisotropy, copying the structure of bone. *Figure 1* shows the typical oriented structure of Scorim molded SEVA-C. The use of Scorim permitted the possibility of obtaining simultaneously higher values of stiffness, strength and ductility (results of tensile tests).

The objective of matching the mechanical properties of human cortical bone was accomplished for 30% HA composites processed by TSE + Scorim. Due to the in-situ formation of HA fibers during Scorim molding, a maximum stiffness of 7.45 GPa (ε_r = 18.7%) was obtained for SEVA-C + 30% HA composites. For SCA + 30% HA a maximum stiffness value of 8.63 GPa (ε_r = 3.1%) could be obtained. The obtained results depend greatly on the type of reinforcement (the stiffer Ca-P glasses creating better results) and on its

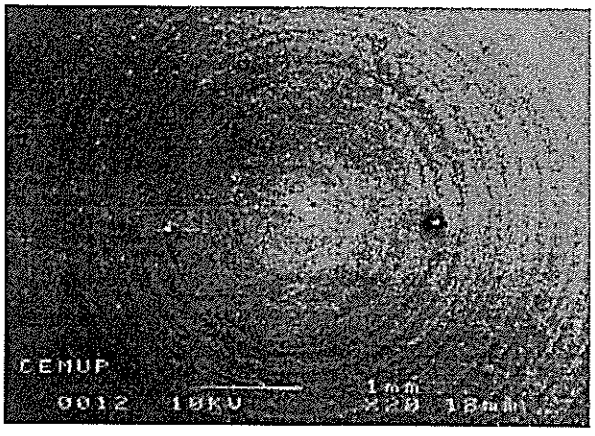

Fig. 1. Typical microstructure of a cross-section of a Scorim molded SEVA-C, presenting clear orientation rings.

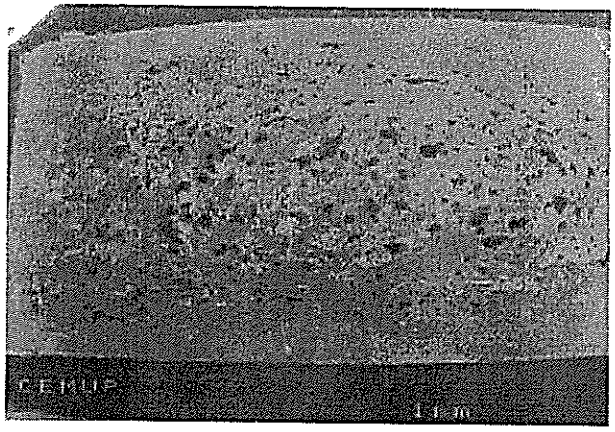

Fig. 2. Structure of a foamed SEVA-C material, evidencing a compact surface and a porous core resulting from the action of the blowing agent.

granulometric distribution (smaller particles give rise to much stiffer composites). Further information on the mechanical properties of the composites may be found elsewhere (6).

Furthermore, it was possible to process biodegradable materials with a compact surface and a porous core, with overall properties similar to the correspondent dense moldings. The typical structure of this type of material is presented in *Fig. 2*. This result, which is due to an additional holding pressure effect generated by the foaming reaction, may allow for the development of materials with the required initial stiffness, and which will after some time start to degrade, allowing bone ingrowth into its porous interior, with any required complex shape (injection molding).

All the molded materials could be EtO sterilized without any meaningful alteration of structure or properties. *Figure 3* presents the degradation of the modulus of SEVA-C and its composites after two consecutive sterilization cycles. These two consecutive cycles were aimed at simulating very drastic EtO sterilization conditions, as with a typical sterilization cycle no changes could be observed. It can be seen that some degradation has occurred. The same was true for UTS (decreasing) and ε_r (increasing). This degradation was due to an amorphization of the polymer, as detected by Raman spectroscopy.

Due to the material's water-uptake capability it was possible to develop bioactive composites for filler amounts of only 30% (wt.) as shown in *Fig. 4*. This was especially clear for composites reinforced with bioactive glasses that could form a Ca-P layer on its surface after 14 days immersion in SBF, with a Ca/P ratio between 1.5 and 1.7 and being composed by tricalcium-phosphate and HA (as identified by thin-film X-ray diffraction). This result means that these materials will eventually be able to bond to bone when implanted. For the non-bioactive materials it has been possible to grow a Ca-P layer on its surface by applying an adapted biomimetic route (see *Fig. 5*).

The degradation behavior has also proved to be greatly dependent on the polymer grade, and on the addition of proteins, as they tend to increase the degradation rates. It was also found that an increase in the HA (or Ca-P glasses) concentration led to a faster degradation rate in simulated physiological solutions, indicating that both the matrix and the reinforcement are being degraded by the body fluids.

Figure 6 shows typical degradation surfaces of SEVA-C (*Fig. 6b*) and SCA (*Fig. 6c*) after immersion for 72 h in a physiological media containing proteins. Further details on the material's degradation have already been published (2).

The materials also present a very interesting, and quite unusual for biodegradable polymers, biological behavior. Neither SEVA nor SEVA + HA composites have shown any cytotoxic effects in vitro cell cultures (*Fig. 7*). Results of standard MEM (minimal essential medium) extraction 72 h tests carried out with mouse

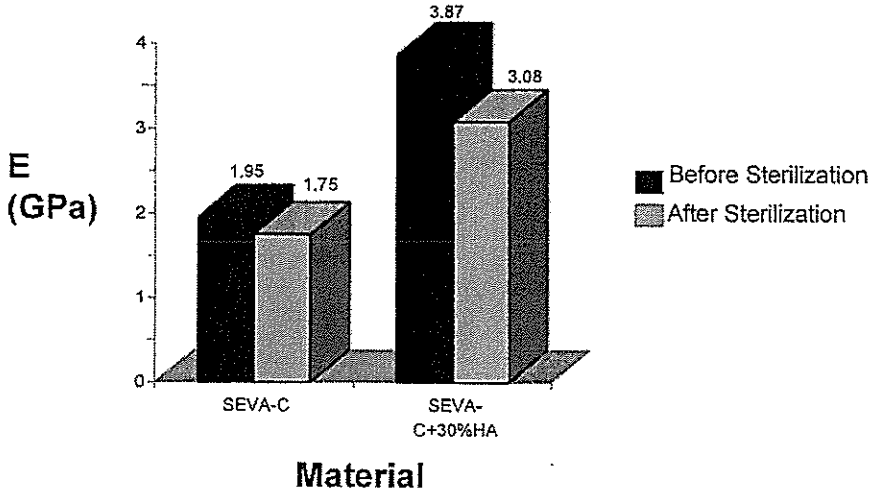

Fig. 3. Degradation of the mechanical properties (an example for the modulus at 1% strain) of SEVA-C and SEVA-C + 30% HA after two consecutive cycles of EtO sterilization. After one sterilization cycle no statistically significant changes could be observed.

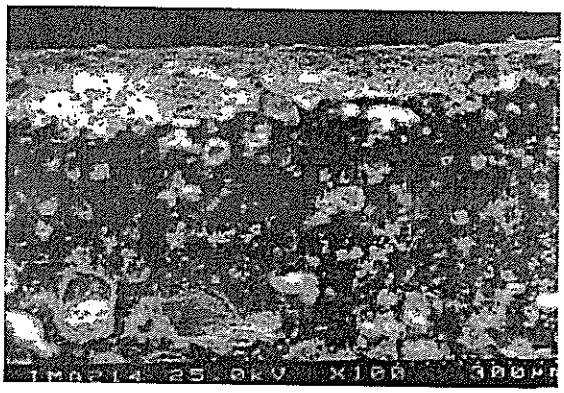

Fig. 4. Cross-section of a bioactive glass reinforced SEVA-C composite that has formed a bioactive layer on its surface after 14 days immersion in SBF.

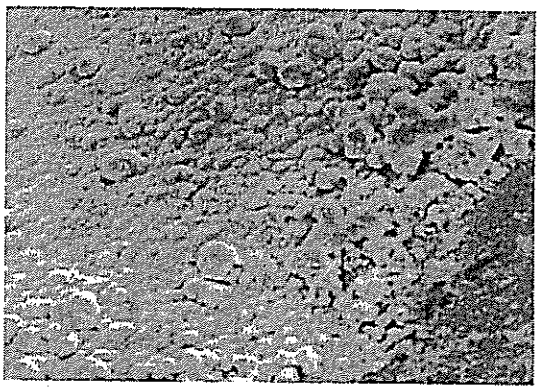

Fig. 5. Biomimetic Ca-P coating formed on SEVA-C, with a typical Ca/P ratio on the HA range.

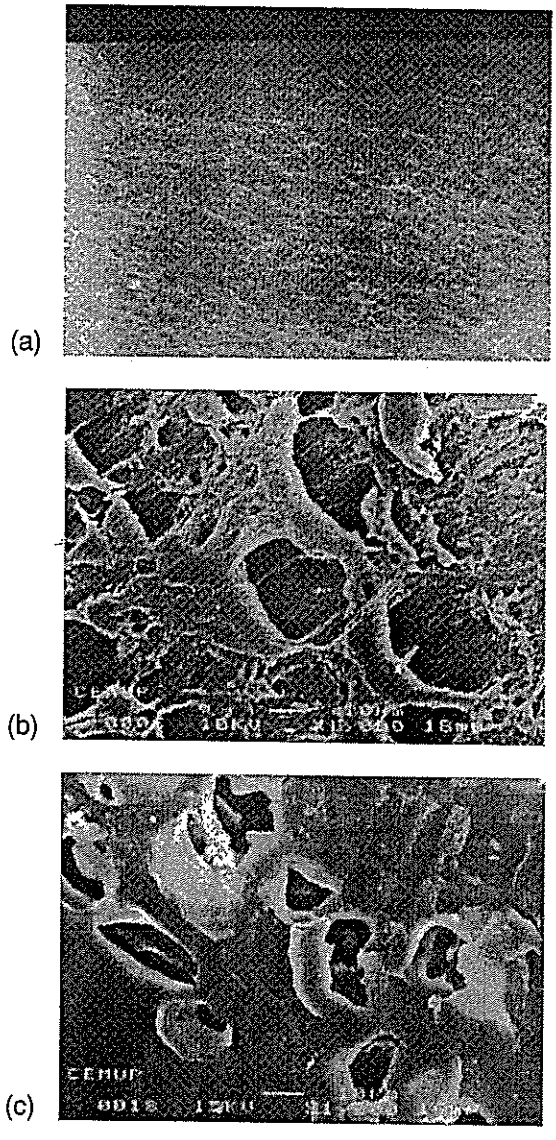

Fig. 6. a) As processed surface of a starch-based blend and b) SEVA-C and c) SCA degraded for 72 h in a physiological media containing proteins.

lung L929 cells clearly show that SEVA-C and its composites are not cytotoxic. In fact, they evidence the same type of behavior as the negative control. On the contrary, SCA shows only a positive response after 10 days of purification treatment (i.e. a pre-extraction in order to remove all the low molecular weight components). This means that processing of these blends should be carried out with extra care in order to avoid any thermal degradation of these very sensitive blends.

The MTT quantitative assays measure the relative formation of formazan by the cells. This is basically proportional to the quantity of cells with an intact respiratory chain (that are with a normal mitrochondrial activity). Results of the MTT 72 h tests (see *Fig. 8*), that were carried out with two different cell lines, mouse lung and human skin fibroblasts, show that

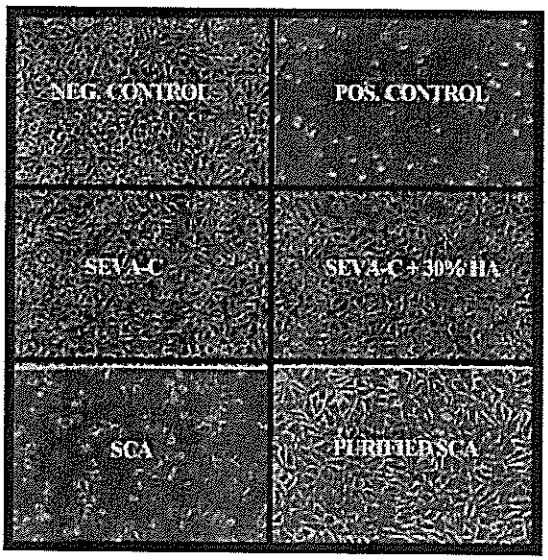

Fig. 7. Results of standard MEM extraction 72 h tests carried out with mouse lung L929 cells. SEVA-C and its composite are not cytotoxic (same behavior as the negative control), while SCA only shows a positive response after purification.

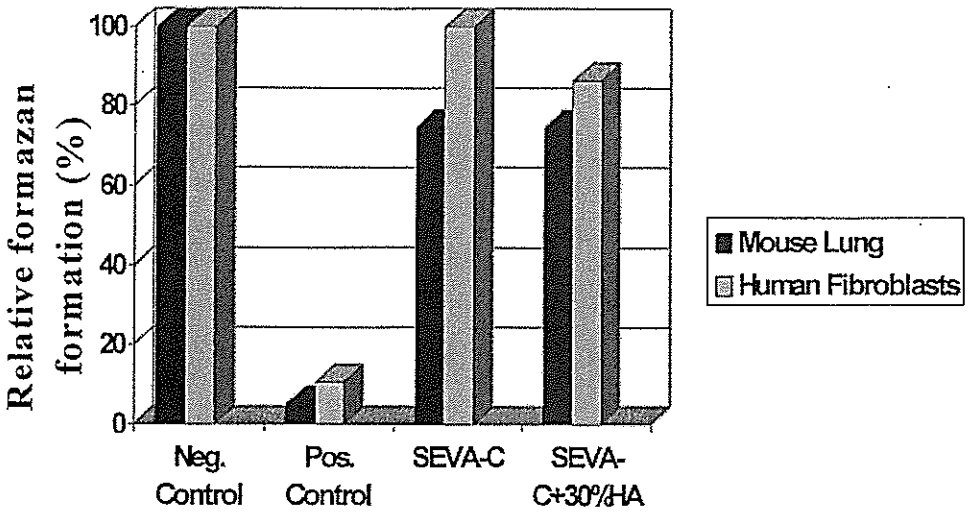

Fig. 8. Results of the MTT 72 h quantitative cytotoxicity assays. Tests were carried out with two different cell lines, mouse lung and human skin fibroblasts. With both types of cells, SEVA-C and SEVA-C + 30% HA evidence a relatively high formation of formazan.

for both types of cells, SEVA-C and SEVA-C + 30% HA evidence a relatively high formation of formazan corresponding to a non-cytotoxicity behavior (usually a value higher than 70% corresponds to a safe behavior). Furthermore, these materials, when implanted in muscle and bone tissue (*in-vivo* goat implantation studies) did not induce to any adverse reactions and they were degrading in-vivo. These biological behavior associated with the mechanical perform and degradation pattern of the materials make them excellent candidates for bone replacement applications.

4. CONCLUSIONS

Novel starch based blends reinforced with bone-like ceramics present a very interesting combination of properties that may allow for their use in temporary orthopedic applications or as tissue engineering scaffolds. It is possible to engineer these materials in order to process composites with an adequate morphological development, which copies not only the structure of bone, but also its mechanical performance. Furthermore, the materials are (or can be made to be) bioactive and present an adequate degradation behavior. Finally, the materials can be EtO sterilized and they present a very good (and quite unusual for biodegradable polymers) biological behavior, as observed on cell cultures and animal implantation studies.

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