Design and Processing of Starch Based Scaffolds for Hard Tissue Engineering

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The design and processing of appropriate porous 3-D scaffolds is one of the most important steps towards the regeneration of damaged tissues/organs using a tissue engineering approach, since most of the cell types require an adequate support in order to form the intended new tissue. This work reports the development of several processing techniques that have been specifically designed for producing biodegradable scaffolds from a range of starch based polymers. The developed methods include melt based processing technologies (based on injection molding and extrusion using blowing agents), combined techniques based on solvent casting and on compression molding associated to particle leaching. It has been possible to produce scaffolds that combine an appropriate degradation rate, with controlled porosity and adequate pore sizes, as well as tissue matching mechanical properties. Furthermore, the developed methods have no negative effect on the biocompatible behavior of the starch based polymers.

INTRODUCTION

Tissue loss or end-stage organ failure resulting from an injury or disease is a major health problem, since the transplantation of tissues or organs in these patients is severely limited by donor scarcity and by the risk of rejection and disease transfer (1–4).

Tissue engineering has emerged as a promising alternative approach to circumvent the limitations of the existing therapies for the treatment of malfunctioning or lost organs (1–6).

In this approach, a porous material acts as a temporary scaffold, serving as an adhesive substrate for the implanted cells and simultaneously supporting the formation of the new tissues/organs. Transplanted cells adhere to the scaffold, proliferate, secrete their own extracellular matrices (ECM), and stimulate new tissue formation. During this process, the scaffold gradually degrades and is eventually eliminated (3, 4).

In tissue engineering of bone, the scaffold matrix must serve an additional function (7–9): it must provide sufficient temporary mechanical support to withstand in vivo stresses and loading. In this case the material must be designed with a degradation rate that ensures that the strength of the scaffold is retained until the tissue-engineered transplant is fully remodeled by the host tissue and can assume its structural role (7–9).

As a consequence, the search for improved biodegradable polymers and for processing techniques to produce scaffolds for hard tissue regeneration, so that physical and chemical properties can be simultaneously optimized, is still an important and very demanding issue in hard tissue engineering research (10–11).

MATERIALS AND METHODS

Usually, polymer scaffold processing is divided in two general groups of techniques: melt processing and solvent processing. Melt processing involves heating the polymer above the glass transition temperature (Tg) or the melting temperature (Tm) and depends on melt viscosity. Solvent processing depends on the polymer solubility in various organic solvents and on the solvent volatility.

This section describes several processing methods for producing the scaffolds based on starch based blends with distinct synthetic polymers, namely with cellulose acetate (SCA), ethylene viny alchool (SEVA-C) and polylactid acid (SPLA), all provided by Novamont,

Italy. Several blowing agents (BA) were selected for the study. However, in this manuscript, we report only results obtained with the blowing agents that produced the scaffolds with higher porosities and/or best interconnectivity, namely: Hydrocerol BIH 40 (BA1), from Clariant, Germany, and Celogen 780 (BA2) from Uniroyal Chemical.

Extrusion and Injection Molding with Blowing Agents:

The polymeric materials were mixed in a rotating drum with one of the blowing agents described above, in amounts from 1% to 15% prior to processing by injection molding or extrusion. The injection molding process used a Krauss Maffei KM60-120A) injection molding machine with a mold that was particularly designed for this application in order to allow maximal expansion and therefore enhance the formation of pores within the poymer melt. In the extrusion process, a Carvex twin-screw extruder with a die diameter of 12 mm was used.

Compression Molding—Particle Leaching:

The starch based polymers and the leachable salt particles of different sizes were blended and then compression molded into a desired shape. The resultant sample was then immersed in water to dissolve the salt particles, creating a porous structure. The leaching procedure was optimized for "excess leaching" of the salt particles, which corresponded to the immersion of each sample in distilled water during 5 days, changing the water daily.

Solvent-Casting/Particle-Leaching:

The polymers were dissolved in an appropriate organic solvent and then mixed with salt particles of different sizes. When the mixture had solidified, by evaporation of the solvent, the samples were immersed in water to leach out the salt particles.

Materials Characterization:

The porous structure of the materials developed was characterized by scanning electron microscopy (SEM), in a Leica Cambridge S360. All the samples were previously gold coated in a Jeol JFC 1100 sputter coater. The SEM analysis allowed us to evaluate the morphology of the pores, their size and distribution and also the interconnectivity between these pores.

The materials were mechanically tested on compression experiments in an Instron 4505 universal mechanical testing machine, using a load cell of 50 kN. Compression testing was carried out at a crosshead speed of 2 mm/min (4.7×10^{-5} m/s), until obtaining a maximum reduction in sample height of 60%. A minimum of six samples of each type was tested.

The degradation behavior was assessed after several pre-fixed aging periods (0, 3, 7, 14 and 30 days), in an isotonic saline solution (NaCl 0.154 M), at 37°C. At the end of each degradation period, the samples were removed from the solution, rinsed with distilled water and weighed, to determine the water uptake. Finally 5 of these samples were dried to constant weight (6 days at 60°C) in order to determine the final dry weight loss and other 5 samples were dried at room temperature and then mechanical tested (as described above) in order to evaluate the changes in mechanical properties after degradation.

RESULTS AND DISCUSSION

Extrusion and Injection Molding with Blowing Agents:

The porosity of the samples obtained by these processes results from the gases (mainly CO_2) released by decomposition of the blowing agents during processing. Therefore, it is difficult to accurately control the porosity and the pore size obtained. However, the consecutive optimization of the processing conditions as well as progressive better selection of the blowing agent and mold design resulted in significant improvements in the percentage of porosity and interconnectivity of the porous structures. This is shown, for example, in *Fig. 1* that displays a representative SEM microphotograph of the structure of a scaffold obtained by injection molding of SCA with only 1.5% of the BA2 (Celogen 780). The estimated porosity in this case is about 40–50%, depending mainly on the type and amount of blowing agent used. In addition, the injection molding and the extrusion processes produce a uniform microporosity throughout the pore walls of the scaffolds (see *Fig. 2*) which can perform an important role in the transport of nutrients during cell culturing, enabling the proliferation of cells also within the scaffolds and not only on the surface.

The mechanical properties of these scaffolds are mainly affected by the synthetic component of the starch based blend used and obviously on the percentage of porosity of the scaffold (see *Table 1*).

Compression Molding—Particle Leaching:

The compression molding and particle leaching technique gives rise to structures on which the porosity and pore size are mainly dictated by the amount and sizes of the leachable particles used and usually with good interconnectivity between pores. *Figure 3* shows the structure of a scaffold based on SCA obtained by compression molding with 65% of salt particles. In this case, about 65% porosity was obtained, and the pore sizes were between 10 to 500μ. The compressive strength of these scaffolds is also deeply dependent on the porosity obtained, as shown in *Table 1*.

Solvent Casting—Particulate Leaching:

The solvent casting and particle leaching method, as in the compression molding with particle leaching, allows for the accurate control of the pore size, distribution of pore sizes and porosity (volume of voids) as

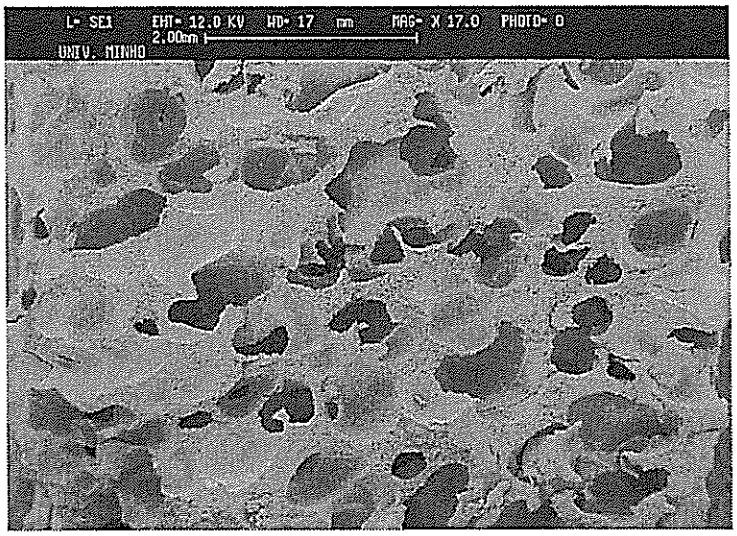

Fig. 1. Scaffold obtained by injection molding of SCA with 1.5% of blowing agent 2 (Celogen 780).

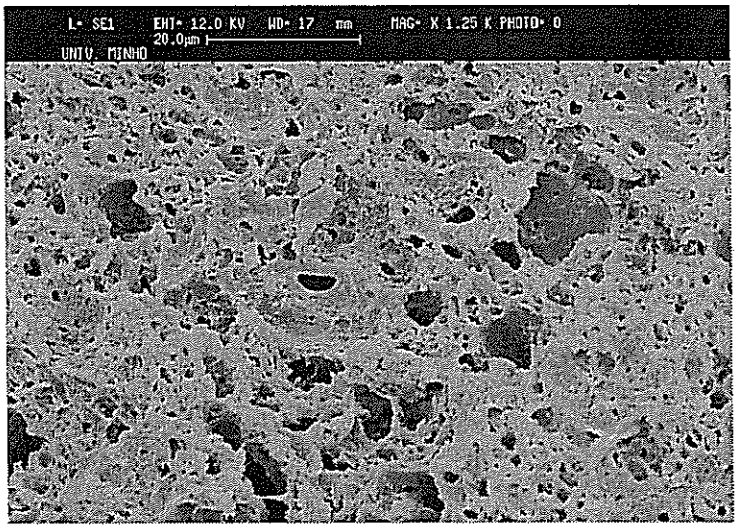

Fig. 2. Scaffold obtained by injection molding of SCA with 1.5% of blowing agent 2 (Celogen 780) showing the uniform microporosity of the structure.

Table 1. Mechanical Properties of the SCA (and SEVA-C) Based Scaffolds Obtained by the Different Methodologies.

Processing method	Type and percentage of blowing agent or porogen used	Compressive modulus (MPa)	Compressive strength (MPa)
Extrusion	1% Hydrocerol BIH 40 (BA1)	240.1 ± 62.8*	17.28 ± 1.7*
	2% Hydrocerol BIH 40 (BA1)	248.9 ± 39.1*	17.5 ± 1.9*
	3% Hydrocerol BIH 40 (BA1)	249.1 ± 85.1*	18.9 ± 1.1*
	2% Hydrocerol BIH 40 (BA1)	124.6 ± 27.2	8.0 ± 0.9
Injection molding	Celogen 780 (BA2)	134.5 ± 39.5	18.4 ± 2.8
Compression molding	50% of NaCl particles	341.6 ± 34.3	67.69 ± 6.2
	65% of NaCl particles	133.7 ± 20.6	20.56 ± 6.2
Solvent casting	60–70% of NaCl particles	170.5 ± 16.09	21.73 ± 1.1

*These values refer to SEVA-C based scaffolds; all the other values presented in this table refer to SCA based scaffods.

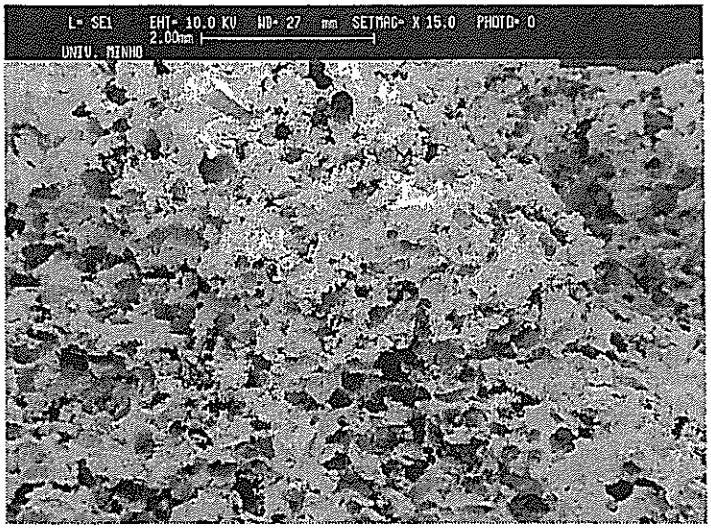

Fig. 3. Scaffold based on SCA obtained by the compression molding and particle leaching method using 65% of salt particles.

these parameters can be tailored by varying the size, shape and distribution of the particles and the chosen volume ratio of polymer/particles. Furthermore, a good interconnectivity between the pores throughout the whole structure can be achieved. However, and as expected, the mechanical properties are lower when compared to the properties of the samples obtained by melt-based technologies (see *Table 1*).

GENERAL REMARKS

In *Figs. 4* and *5*, the water uptake and degradation behavior of the scaffolds obtained by the different processing methods described in this study may be compared. The scaffolds obtained from the above described methods presented similar degradation profiles. However, they exhibited different degradation rates, according to processing method and processing conditions, especially those that have direct influence in the percentage of porosity, such as the amount of blowing agent and/or amount of leachable particles. In general, the methods that give rise to scaffolds with higher porosity and interconnectivity exhibit higher water uptake. However, the degradation rate is not only influenced by the porosity of the structure but also by other aspects related to the processing method. For example, the scaffolds obtained by compression molding and particle leaching exhibit higher water uptake than those obtained by extrusion and injection molding with blowing agents, but their weight loss is lower. This is most probably due to the thermo-mechanical degradation undergone by the materials processed by injection molding and extrusion, which leads to an easier breakdown of the polymeric chains. In addition to this, the lower porosity and poor interconnectivity of the samples obtained by these processes, but particularly with the injection molding, may contribute to faster degradation rates due to enhanced autocatalysis in those scaffolds, which are unable to evacuate acidic degradation by-products.

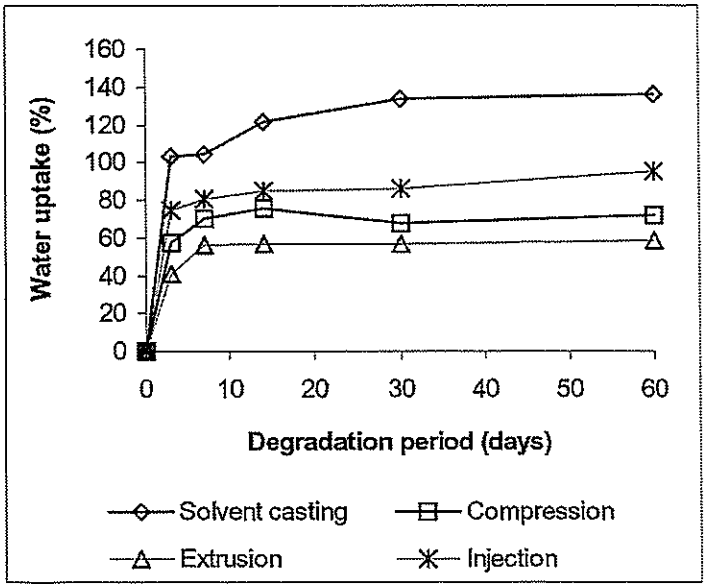

Fig. 4. Water uptake vs. degradation period for SCA based scaffolds obtained by the different processing technologies developed. Standard deviations are between 3.1 to 5.36% (not shown for easier visualization of the displayed results).

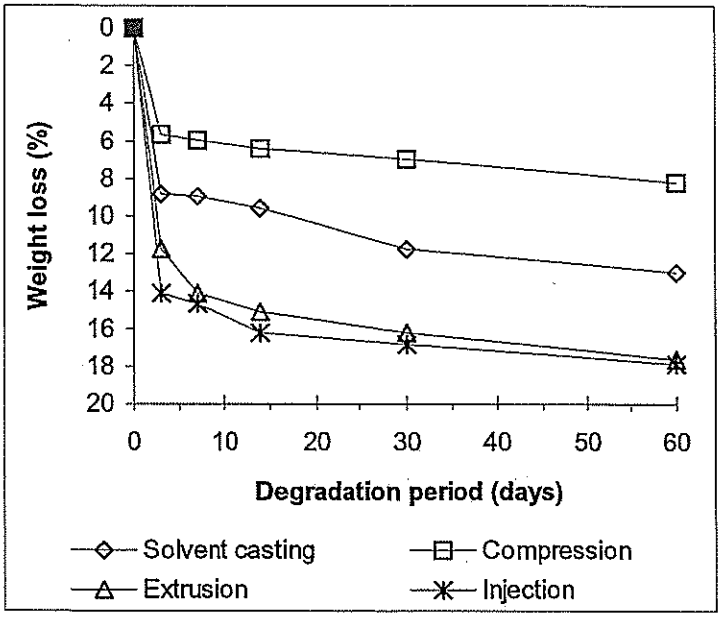

Fig. 5. Weight loss vs. degradation period for SCA based scaffolds obtained by the different processing technologies developed. Standard deviations are between 0.38 to 1.18% (not shown for easier visualization of the displayed results).

The mechanical properties of the scaffolds do not exhibit signifficant decreases after in-vitro degradation during the first 30 days as shown, for example, for SPLA based scaffolds obtained by extrusion and compression molding (see *Fig. 6*). The mechanical properties of these scaffolds, namely the compressive modulus, are in the range of those reported for human trabecular bone, and the fact that it is decreased less than 30% in the first 30 days of in-vitro degradation is determinant for their application as bone tissue engineering scaffolds.

The biological behavior of starch based polymers has been the subject of several different *in vitro* and *in vivo* studies (12–14). All these studies have shown that these polymers exhibit a biocompatible behavior, which is not affected under controlled processing conditions. More recently, preliminary cell culture studies have demonstrated that the starch based scaffolds produced by the methodologies described herein allow for the proliferation and growth of human osteoblasts (15). Although it is necessary to further test these materials with respect to their ability to act as a support for the growth and differentiation of cells, these preliminary results indicates the potential of the developed porous materials for use as bone tissue engineering scaffolds.

CONCLUSIONS

Several processing methods to obtain starch based scaffolds were developed. These methodologies allow us to tailor, to an extended degree, the pore sizes and

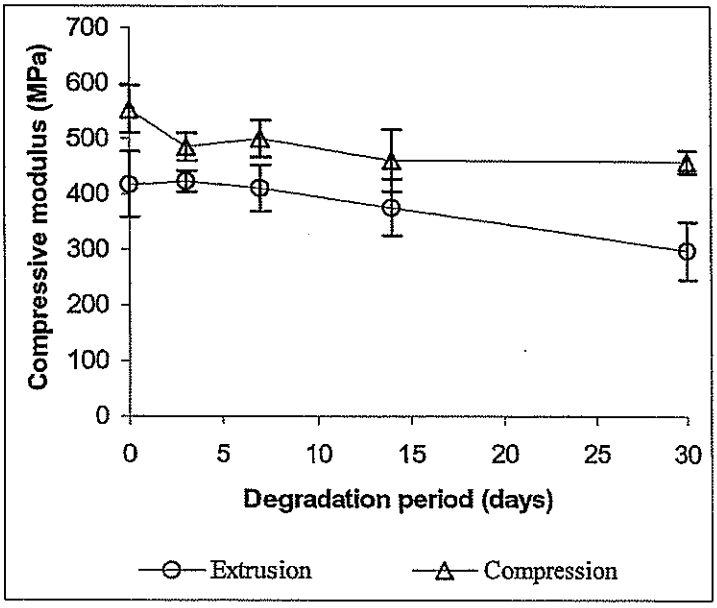

Fig. 6. Compressive modulus of SPLA based scaffolds obtained by two different processing technologies after several degradation periods in a saline solution.

pore structure of the scaffolds as well as their degradation rates since it was shown that the degradation rates can be significantly different, depending on the processing method and on the final porosity obtained.

Furthermore, although only few data on mechanical properties of scaffolds for tissue engineering is found in the literature, it is possible to conclude that mechanical properties of all the tested scaffolds are very promising, when compared to scaffolds obtained from other biodegradable polymers. In addition, these properties are not signifficantly affected in the first 30 days of *in vitro* degradation, which suggests that the scaffold will be able to provide the necessary structural support in the first period of implantation.

In conclusion, all these results, from both a materials science and a biological perspective, are very promising for the future application of starch based biodegradable polymers as tissue engineering scaffolds.

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