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MORPHOLOGY AND MECHANICAL PROPERTIES OF PLLA AND PCL SCAFFOLDS

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ABSTRACT

Human tissue engineering, comprising methods and tools to create implants, is a promising although as yet a very underdeveloped field of research into the regeneration of specific damaged or necrotic tissue. Porous scaffolds play an important role in tissue engineering. The porous cell culture scaffolds in this study were produced through thermally induced phase separation (lyophilization). This technique yields considerable variations in scaffold microstructures (pore size and morphology) as a function of the polymer, solvent and thermal processing. PLLA and PCL were used with chloroform, 1,4-dioxane and water as solvent. We observed a decrease in mechanical properties with increasing pore size in the two polymers under study. However, we found that PLLA, which possesses larger pore sizes than PCL, showed superior mechanical properties, which we explain in terms of crystallinity.

Keys: scaffolds, liophilization, morphology, mechanical, properties, thermal, process

INTRODUCTION

The replacement of damaged or necrotic tissue due to infection, trauma, tumor resection or congenital defects remains a very underdeveloped area of research, but of great interest in many fields of medicine (Trauma/Orthopedic Surgery, Rheumatology,

Pediatrics, Clinical Genetics, among others). In certain situations, for example, native bone tissue is unable to generate the amount of new tissue that is needed to achieve the therapeutic goal. The "Gold Standard" of current treatment strategies is based on the use of (autologous or autograft) tissue grafts of an individual's own tissue, which represents an ideal substitute. However, the number of autografts that any one patient can undergo is limited, and their preparation involves an added risk of morbidity caused by the consequent extractive surgical procedures, which are commonly associated with risks of infection, hematomas and chronic pain.

These disadvantages can be avoided by using allografts (obtained at the time of an organ donation). Their advantages over autografts include greater availability and the absence of any secondary surgical site that can be a source of discomfort on the patient for years. However, allografts have other limitations, such as the risk of disease transmission and immunogenic reactions [1, 2].

A promising alternative to auto- and allografts is the use of surrogate tissues obtained by Tissue Engineering. Tissue engineering is an interdisciplinary area of regenerative medicine that combines the principles of engineering and biology associated with biomaterials, to obtain a functional tissue *in vitro* that can be transplanted to an individual who has suffered tissue loss. Extracting a patient's own stem cells (adult) and subsequent expansion in the laboratory (*in vitro*). The process is as follows:

• Extract patient's own stem cells (adult) and subsequent expansion in the laboratory (*in vitro*).

• Plant in a support or "scaffold" in the presence of recombinant factors that promote cell differentiation to grow the desired tissue.

• Implementation of the support with the individual recipient tissue to replace the damaged or missing tissue.

The vast majority of three-dimensional structures for applications in the field of tissue engineering are made from polymeric materials. The most commonly used polymers are typically straight chain aliphatic polyesters such as polylactide and polyglycolide PGA PLLA and their copolymers, which are degraded by the hydrolysis of their ester groups. Another commonly used polymer is polycaprolactone the degradation of which requires a longer time period.

Tissue Engineering requires three-dimensional structures that should share the following characteristics: biocompatible and biodegradable materials with a controllable resorption rate to match the growth of tissue cells *in vitro* and *in vivo*, which are three dimensional, which possess a high degree of interconnected porosity for cell growth and nutrient transport, which have a suitable surface for access, proliferation, and differentiation of cells, and which possess similar mechanical properties to the tissue that is to be replaced [3-6].

In this paper, thermal induced phase separation (lyophilisation) was employed to prepare PLLA and PCL scaffolds. There are many works in the literature on the fabrication of scaffolds, but none examines such important factors as the influence of the solvent, thermal treatment, the polymer, and the study of its mechanical properties.

EXPERIMENTAL PROCEDURES

Materials

Optically pure poly(l-lactide) containing less than 0.01% of residual solvent and less than 0.1% residual monomer was supplied by Biomer L9000(Germany). Polycaprolactone was supplied by Purac Biomaterials Purasorb PCL 12 (Holland) [7].

The solvent, 1,4-dioxane, chloroform (Panreac p.a. Barcelona, Spain), was distilled by conventional methods. Distilled water (Panreac p.a., Barcelona Spain) was used without further purification.

Scaffold fabrication

Porous scaffolds were prepared with 2.5% (w / v) of PLLA and PCL in 1,4-dioxane and chloroform both pure and with 0, 1, 2, 5 and 10% distilled water. The mixture was stirred at 50 ° C for 2 h to obtain a homogeneous polymer solution. The polymer solutions were then poured into aluminum molds specially built for the manufacture of these scaffolds and chill
ed to -60, -15 and 0 ° C. The samples were freeze-dried at -62 ºC and 0.5 mmHg for 7 days to remove the solvent. The scaffolds were fabricated using a phase-separation technique.

Mercury pycnometry

The porosity of the scaffolds was quantified by mercury pycnometry. To do so, the scaffolds were dipped one by one in a container of mercury placed on electronic scales with the help of a metal device. Knowing the density of mercury (ρ_{HG} = 13.57 g/cm^3) and the mass indicated by the scale, we may calculate the volume of the mercury (Vol_{Hg}). The volume displaced by the mercury is equivalent to the volume of the sample in question. So, knowing the initial mass and Vol_{Hg} thereof (M_{sa}), the bulk density (ρ_a) may be calculated with the following equation:

$$\rho_a = M_{sa} / Vol_{Hg} \qquad (1)$$

Using both bulk density and the density of the polymer (ρ_p), measured by pycnometry with the pulverized material, the percentage porosity was calculated by the following equation:

$$\% P = (1- \rho_a/\rho_p) \times 100 \qquad (2)$$

where, P is the percentage porosity. Measurements were made for each material.

SEM analysis

The bulk morphology of the scaffolds was examined using scanning electron microscopy (SEM) (HITACHI S-3400N, Tokyo, Japan). Prior to analysis, the samples were coated with a layer of gold, in a JEL Ion Sputter JFC-1100 at 1200 V and 5 mA., to avoid sample charging under the electron beam.

DSC analysis

The thermal characteristics of the polymer were determined using differential scanning calorimeter (DSC TA Instruments) equipped with an intracooler. Approximately 10 mg of polymer was placed in a crimp-sealed DSC hermetic aluminum pan. A nitrogen purge gas was used to prevent oxidation of the samples during the experiments, which

were subjected to temperature scans ranging between -20 ºC and 200 ºC at temperature/time ratios of 10 ºC/min.

Mechanical property

Mechanical testing of the pore scaffolds was performed using a Universal Testing Machine (Instrom, Model: 4502 UK). The PLLA and PCL scaffolds preparation procedure was the same as described for the fabrication methods. The diameter of the scaffold disk was 11 mm and its thickness was 2 mm. The compressive modulus was defined as the initial linear modulus and the yield strength was determined from the intersection of the two tangents on the stress-strain curve around the yield point. Four scaffolds were mechanically tested for each sample.

RESULTS AND DISCUSSION

We observed that the morphology and size of the pores varied in this work, depending on the solvent, the temperature at which phase separation occurred, and the type of polymer that was employed.

Influence of solvent

The three dimensional structures obtained using the chloroform solvent, in the two polymers under study, had a morphology and irregular pore size and porosity with a percentage lower than 50%, and the appearance was that of a porous film. This is due to the low melting temperature of the chloroform at -63.5 ° C compared with that of 1,4-dioxane that melts at 11.8 ° C. When the polymer solution was frozen at -60 ° C, not all the settlement was frozen and we proceeded to freeze it instead of sublimating it to evaporate the chloroform, hence the appearance of porous film obtained for both the PLLA and for the PCL, as we can see in Figure 1.

The solvent of 1,4-dioxane, still provided higher porosity and uniform pore size, in addition to a good level of interconnectivity [8]. This level is of great importance, as

tissue growth in relation to vascularization and nutrient distribution throughout the tissue that has formed are both dependent on that same parameter (see Figure 2).

The addition of water to the solvent has a great influence on the morphology of the scaffolds and mechanical properties. Scaffolds prepared with a mixture of solvent/ distilled water at 1, 2, 3, 5 and 10% water showed less consistency and worse mechanical properties. SEM observation indicated the presence of pores with broken and poor cohesion between the layers of scaffolding for the two polymers under study (see Figure 3 and 4). This can be explained by the fact that when the amount of non-solvent (water) increases, the polymer-solvent interaction is less and may induce the formation of a polymer-poor phase, which favors phase separation as liquid-liquid, reducing the solubility of the polymer in thedioxane / water or chloroform / water mixtures [8-10]. However, other authors (Park et al) have found that the presence of water favors a dual pore system with different pore sizes of 200-300 microns in large pores and of 5-20 in small ones. However, with our manufacturing technique, we need not use water or any other additional operation for a dual pore system (see Figure 5). Besides the water (in any ratio 1, 3, 5, 10%) is caused by the lack of cohesion in the scaffold and the break-up of the pores, making them unsuitable for cell culture.

The lyophilization technique is based on thermodynamic demixing of a homogeneous polymer-solvent solution into a polymer-rich phase and a polymer-poor phase, usually by cooling the solution below a bimodal solubility curve. Solvent is removed by freeze drying, leaving behind the polymer as a foam. Pore size depends on the solvent cristal formed. The morphology is controlled by any phase transition that occurs during the cooling step: liquid- liquid or solid- liquid. The liquid- liquid phase separation leads isotropic forms with highly interconnected pores and the solid- liquid phase separation results in anisotropic foams with a sheet like morphology.

Thermal treatment

We prepared three different thermal treatments. Once the polymer solution was obtained, it was cooled to -60, -15 and 0 - 5 ° C and then at -62 ° C and it then

underwent lyophilization. The results obtained for the two polymers can be seen in Table 1.

In this table, we can see that the PLLA has a larger pore size for the three tempering temperatures under study. PCL has a smaller pore size, although there are applications that require a smaller pore size, such as bone repair implants [11, 12]. In both cases the polymers had a porosity of over 95% and good interconnectivity in their pore systems (see Figure 6 and 7).

We have found that the porosity of the scaffolds prepared by the lyophilization process depends on the cooling rate of the polymer solution. Quenching generally produces a reduction of pore size in the case of a high cooling rate, the two phases that form are rapidly frozen and smaller pores are formed. Conversely, if the speed is slower, the phenomenon of coalescence reduces the interfacial energy and produces larger pores. In our case, the latter applies as scaffolds with larger pores are those that have been tuned to 0 °C.

The hardening temperature is also of great importance as it directly affects solvent crystallization [11-13]. When the hardening temperature is low compared to the crystallization solvent temperature, the solvent crystallizes very rapidly and produces a separation of solid-liquid phases. The scaffolds obtained in this way have a very anisotropic tubular morphology with an internal structure similar to that of a ladder. The cavities are parallel to the direction of solidification, with repeated divisions. The progression of crystallization from the solvent front defines the main orientation of the pores, the long axes of which are parallel to the direction of cooling.

Conversely, if the cooling temperature is close to the solvent crystallization temperature, crystallization is slower, thereby favoring the separation of the liquid-liquid phase. If this occurs between the binodal and spinodal curves of the phase diagram, the separation of the liquid-liquid phase is produced by nucleation and a growth mechanism, resulting in a structure that is generally dispersed. In contrast, if phase separation occurs below the spinodal curve, the polymer solution is prone to phase separation by spontaneous liquid-liquid formation of an interconnected network of pores (see Figure 7). Moreover in some cases micropores are formed in the polymer structure [8,9], ie on the walls of the macropores (see Figure 5).

Mechanical property

Scaffolds provide the osteoconductive character that the implant must have, which is achieved through the presence of a porous network that must be formed by interconnecting pores. The mechanical properties are perhaps one of the most difficult requirements to meet. The porous scaffolds must support the mechanical demands of the injury site throughout the regeneration process, which implies that the properties and degradation mechanisms must work in harmony with the formation of new tissue. This will mainly depend on the material properties of the regenerative tissue that the patient requires.

The mechanical properties of PLLA and PCL scaffolds with three different pore sizes were evaluated. All the scaffolds in our study had a porosity of above 90%. In figures 8, 9, 10 and 11, we can see how the compressive modulus and the yield stress are heavily dependent on pore size, both for PLLA porous structures and for the PCL. The lower mechanical properties of the scaffolds with larger pore sizes could be consistent with the reduced polymer content[13-15]. For the two polymers under study, we observed that the mechanical properties decreased as the pore size increased. However, we found that the PLLA which possesses larger pores than PCL, had superior mechanical properties to the same polymer composition, due to crystallinity. The scaffolds produced with semicrystalline polymers were more porous (because the structure was more organized), less dense, with a different pore morphology and their mechanical properties depended strongly on crystallinity. In summary, we can say that two different polymers with a similar pore size, and with the same solvent concentrations, would have comparable mechanical properties, if both had the same crystallinity, and if their pore morphology were the same, which would imply the same phase separation process (solid-liquid or liquid-liquid)[14, 15].

A comprise is needed for the production of scaffolds for bone regeneration between mechanical properties and pore size and morphology, to allow proper adhesion and proliferation of cells under load which is when permeability to nutrient transportation varies most.[16]

CONCLUSIONS

The solvent of 1,4-dioxane, provides greater porosity and uniform pore size, as well as an interconnected matrix of pores. Quenching generally produces a reduction in size of the pores, on the contrary, if the speed is slower, coalescence reduces the interfacial energy and produces larger pores. Regarding the mechanical properties of the two polymers, we observed that their mechanical properties decreased as the pore size increased.

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