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# A study of the mechanical properties and cytocompatibility of lactide and caprolactone based scaffolds filled with inorganic bioactive particles

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ABSTRACT

The mechanical properties of highly porous (90% porosity) poly(L-lactide) (PLLA), poly(ε-caprolactone) (PCL) and poly(L-lactide/ε-caprolactone) (PLCL) were investigated. Young's modulus of non-porous PLLA, PCL and PLCL dropped from 2263.4, 183.7 and 5.7 MPa to 16.8, 1.0 and 1.0 MPa, respectively, for their ~90% porous counterparts. Elongation at break of PCL decreased noticeably with porosity fraction while PLCL maintained a highly elastomeric character and strain recovery capacity even in the presence of pores.

Inorganic bioactive particles (hydroxyapatite or bioglass) were added to confer bioactivity to the aforementioned synthetic bioresorbable polymers, and their effect on the mechanical properties was also investigated. Addition of 15 vol.% of inorganic bioactive particles increased the Young's modulus of highly porous PLLA from 16.2 to ~30 MPa. On the contrary, the difference between Young's modulus of filled and unfilled PCL and PLCL scaffolds was not statistically significant.

Finally, an *in vitro* study of the cytocompatibility and adhesion of adipose derived stem cells (ADSCs) was conducted. The observed viability and excellent adhesion of these cells to both porous and non-porous templates indicate that the employed materials can be good candidates for application in tissue engineering.



## 1. Introduction

Tissue engineering is defined as an interdisciplinary field that applies the principles of engineering and life sciences for the development of biological substitutes that restore, maintain or improve tissue functions [1]. In this field, 3-dimensional scaffolds play a pivotal role because they provide a substrate for cells to attach to, proliferate and form an extracellular matrix. These scaffolds must possess adequate mechanical properties to provide sufficient biomechanical support during the process of tissue regeneration and at the same time, they should have a highly porous structure with well-interconnected pores in order to facilitate the diffusion of nutrients and the removal of metabolic waste [2]. Hence a balance between total pore volume and mechanical strength is required. Finally, the materials used for the fabrication of these scaffolds should also be biocompatible and be degraded at a controllable rate so that the space occupied by the initial scaffold is replaced by regenerated host tissue [3].

Among the numerous materials available for the fabrication of scaffolds, synthetic bioresorbable polyesters – such as polylactides and polylactones – have attracted a great deal of attention for use in tissue engineering because of their biodegradability, biocompatibility and easily controlled (tunable) properties. While poly(L-lactide) (PLLA) behaves like a glassy material at body temperatures, showing high strength, high modulus and low ductility, poly(ε-caprolactone) (PCL) is just the opposite, showing low strength and modulus with large elongation at break [4,5]. To complement the properties of PLLA and PCL for specific medical applications, copolymers of lactide (LA) and ε-caprolactone (ε-CL) have also been proposed. PLCL copolymers, depending on their composition and chain microstructural features, can be tailored to possess different mechanical properties [6,7].

The aforementioned (co)polyesters, however, do not possess the necessary specific bioactive abilities to induce adequate proliferation and differentiation of cells. Therefore, addition of phosphate or silicate based bioactive fillers is being considered as a strategy to impart bioactivity to bioresorbable synthetic polymers in the regeneration of hard and soft tissues [8].

In bone tissue engineering, incorporation of a biocompatible and bioresorbable polymer to bioactive glass or hydroxyapatite scaffolds has

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been proposed as a method for improving the toughness of the ceramic scaffolds [9]. Polymer scaffolds loaded with hydroxyapatite or bioglass particles display improved mechanical properties [10,11] due to the presence of stiff particles in a soft polymer matrix. Moreover, the osteointegration of the scaffold with the surrounding bone is enhanced [12,13] and, according to literature [14–16], *in vitro* growth and osteogenic differentiation of cells seeded on bioglass or hydroxyapatite filled polymer scaffolds are improved with respect to the unfilled counterparts.

While bioactive ceramics have been thoroughly considered for bone repair, little attention has been paid to their application in the regeneration of soft tissues. Recent works [17,18] have demonstrated, however, the ability of bioactive glasses to enhance neocartilage formation during *in vitro* culture and to promote angiogenesis, which is critical for numerous applications such as healing of soft tissue wounds. For the regeneration of soft tissues, soft biomaterials with elastomeric behavior are desirable [19,20]. This is the reason why we studied combinations of bioactive ceramics with thermoplastic materials whose behavior ranged from glassy to elastomeric, covering a wide range of mechanical properties found in real body tissues.

This study focuses on the mechanical properties of highly porous PLLA, PCL and PLCL scaffolds. The mechanical properties were measured by means of tensile tests and the effect of the porosity and bioactive particles content were evaluated using the Gibson–Ashby [21] and Ishai–Cohen [22] approaches. The combination of these two models can predict the tensile modulus for porous scaffolds filled with inorganic bioactive particles.

In addition, an *in vitro* study of the attachment, viability and growth of adipose derived mesenchymal stem cells (ADSCs) on porous scaffolds and non-porous films of PLLA, PCL and PLCL filled with inorganic bioactive particles was conducted. Mesenchymal stem cells are multipotential cells that can differentiate into bone, cartilage, fat, muscle and other types of tissues when induced by the appropriate biological cues *in vitro* [23,24] and therefore, they have been introduced as a new cell source for application in tissue engineering [25,26]. Although these cells have been traditionally obtained from the bone marrow [27] other sources, such as adipose tissue, have recently been proposed. This source of stem cells has a promising future in tissue engineering applications because of the abundance of this tissue in the human body and the ease with which a large number of cells can be obtained from a small amount of tissue [28]. The cytotoxic evaluation of the materials was studied by means of an MTT assay, whereas the adhesion of the ADSCs was investigated by scanning electron microscopy (SEM).

## 2. Materials and methods

### 2.1. Materials

PLLA of a weight-average molecular weight ($M_w$) of 500,000 and dispersity index (D) of 1.56, PCL of a $M_w$ of 120,000 and D of 1.61 and PLCL of LA/CL molar weight ratio approximately 70/30, a $M_w$ of 190,000 and D of 1.67, were kindly supplied by Purac Biochem (The Netherlands).

The 45S5 Bioglass® particles were kindly supplied by Novabone® (US). The composition of Bioglass® (in wt.%) was 45.0% $SiO_2$, 24.4% $Na_2O$, 24.5% CaO and 6.0% $P_2O_5$. The particles had a size <60 μm, a mean particle size of 9 μm and density of 2.75 g $cm^{-3}$. To measure the size distribution of these particles, a dispersion of the particles in ethanol was prepared and after sonicating for 15 min, some drops were placed on a microscope glass slide. Finally, the sample was examined in a microscope and the size distribution was determined using the ImageJ software.

The hydroxyapatite sintered powder was purchased from Plasma Biotal Limited (UK) and its main components (in wt.%) were 55% CaO and 41% $P_2O_5$. The particle sizes were <60 μm in all cases, with a mean particle size of 6 μm and a density of 1.20 g $cm^{-3}$.

### 2.2. Preparation of samples

Porous scaffolds were prepared by a solvent casting/particulate leaching method described elsewhere [29], using chloroform (Panreac, Spain) as solvent and NaCl (Sigma Aldrich, Spain) particles (200–355 μm) as porogen. A dissolution of polymer in chloroform was mixed with salt particles in different ratios in order to obtain scaffolds with various porosities. The bioactive particles were only added to the scaffolds with higher porosities (~90%) so as to reach levels of 5, 10 and 15 vol.%. This mixture was ultrasonicated for 15 min to obtain a homogeneous distribution of bioactive particles in the solution and to avoid the formation of agglomerates. It was then transferred to a square Teflon mold (100 × 100 $mm^2$) and after the evaporation of the chloroform, sheets of ~1.2 mm were obtained. In the case of non-porous films no salt was added during the manufacturing and the sheets obtained had a thickness of ~0.2 mm. Finally, either dumbbell-shaped samples (for mechanical testing) or 6 mm diameter circular samples (for cellular study) were punched out from these sheets and were placed in distilled water for 48 h to leach out the salt particles.

### 2.3. Morphology and phase characterization of samples

Morphology of the scaffolds was studied via scanning electron microscopy (SEM) (HITACHI S-4800). Samples were fractured after being frozen in liquid nitrogen. To make them conductive, they were coated with a 150 Å layer of gold in a JEL Ion Sputter JFC-110 at 1200 V and 5 mA.

Porosity of scaffolds was calculated according to Eq. (1):

$$\%\text{Porosity} = 100\,(1\text{-}\rho_{\text{scaffold}}/\,\rho_{\text{solid}}) \tag{1}$$

where $\rho_{\text{scaffold}}$ is the apparent scaffold density and $\rho_{\text{solid}}$ is the polymer density. In the case of adding bioactive particles, the composite density ($\rho_{\text{solid}}$) was calculated as:

$$1/\rho_{\text{solid}} = \left(\text{wt.}\%_{\text{polymer}}/\rho_{\text{polymer}}\right) + \left(\text{wt.}\%_{\text{particle}}/\rho_{\text{particle}}\right) \tag{2}$$

where $\rho_{\text{polymer}}$ is the polymer density, $\rho_{\text{particle}}$ is the density of bioactive particles (1.20 g $cm^{-3}$ for the hydroxyapatite and 2.75 g $cm^{-3}$ for the

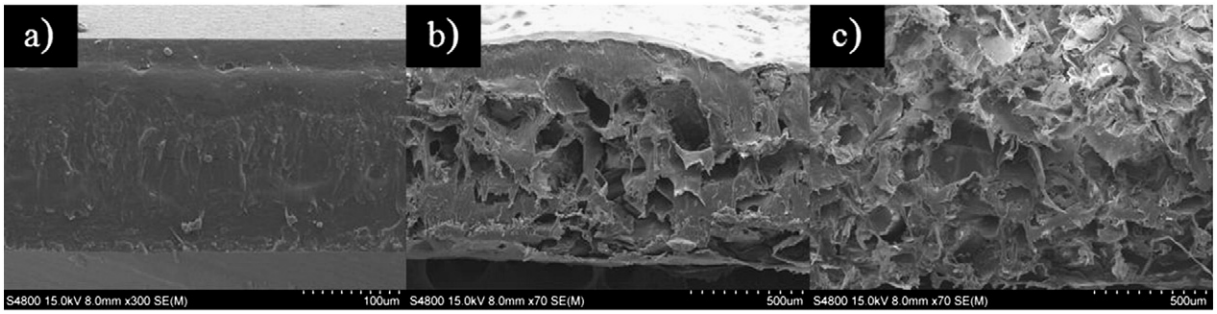


**Fig. 1.** SEM micrographs of a) non-porous PLLA film (PLLA1), b) PLLA scaffold with ~75% of porosity (PLLA3) and c) PLLA scaffold with ~90% of porosity (PLLA5).