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Tuneable hydrolytic degradation of poly(L-lactide) scaffolds triggered by ZnO nanoparticles

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ABSTRACT

In this work we fabricate porous PLLA and PLLA/ZnO scaffolds with porosities ranging from 10 to 90% and average pore diameter of 125–250 μm by solvent casting/particulate leaching method. The structural evolution of PLLA/ZnO scaffolds during their *in vitro* degradation in phosphate buffered saline (PBS) at physiological pH (7.4) has been studied as a function of porosity and obtained results were compared to plain PLLA scaffolds. The changes induced upon the hydrolytic degradation of scaffolds have been explored by measuring the pH changes of the medium, the mass loss, thermal transitions, crystalline structure, thermal stability and the morphological changes. It is shown that the degradation profile of scaffolds could be successfully modified by tuning both the amount of ZnO nanoparticles and by varying the scaffold porosity. Results reveal that the water dissociated on ZnO nanoparticle surfaces initiate hydrolytic degradation reactions by reducing the strength of the chemical bonds of the adjacent PLLA chains, causing them to further divide into water-soluble oligomers. Obtained results may be useful towards the development of antibacterial porous structures with tuneable degradation rates to be used as a substrate for the growth of different kind of cells and tissues.

1. Introduction

Tissue engineering (TE) with porous three-dimensional (3D) biodegradable scaffolds has emerged as a promising method for tissue regeneration [1,2]. Porous scaffolds could recreate biological tissue substitutes that restore, maintain or improve tissue functions [3]. To obtain structurally and functionally developed tissues, these scaffolds used for TE should possess a three-dimensional highly porous structure with open and interconnected pore network that allows cell growth and at the same time permits the flow of nutrients and metabolic waste [4,5]. Scaffolds allow the development of less invasive and resorbable devices that would avoid the need for a second revision surgery to remove the implant, reducing the eventual infection risk for the patient [6,7]. Depending on the intended application, fast or slow degradation of scaffolds is desired since these structures should present a controllable degradation rate that matches the cell/tissue growth. For instance, a slow degradation could yield to stress shielding of the growing tissue and which limits the regeneration process [8], while a fast degraded scaffold does not allow the sufficient development of the neo-tissue [9]. This fact demonstrates the need of extensive works focused on adjusting the hydrolytic kinetics of scaffolds.

Porous scaffolds composed of several biocompatible materials have been already proven to have potential uses for tissue engineering applications [10,11]. Currently, polymer-based scaffolds are the most commonly used materials for the fabrication of these three-dimensional porous architectures [12,13]. In comparison with natural biodegradable polymers, the use of synthetic polymers for TE presents the advantages of their mass production capability, high reproducibility, tailored structural features with custom-made mechanical behaviour and tuneable degradation rate to meet the intended biomedical application [14–16].

Biocompatibility of the scaffold is an essential requirement and the materials used must not elicit any cytotoxic responses during their degradation into the human body. Besides of its already known biocompatibility and biodegradability, polylactides are an environmentally friendly polymer since it could be derived from completely renewable resources and could be ultimately degraded into metabolizable products by natural pathways *via* random-scission of their ester linkages [17,18]. The semicrystalline poly(L-lactide) (PLLA) enantiomer emerges as the ideal candidate as it has shown a good *in vivo* track record [19,20]. This environmentally friendly polymer is currently being widely used as sutures, drug delivery devices and tissue engineering substrates as it possess favourable cell adhesion and proliferation properties. PLLA displays a tuneable degradation rate, good mechanical properties and easy of processing (they can be shaped into screws, scaffolds, pins, plates…) [21]. Moreover, its glass transition temperature (T_g) is located at about 55–60 °C, having therefore a high mechanical modulus at physiologic temperature [22]. However, its further application in tissue engineering

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has been largely limited owing to its slow degradation rate, which makes PLLA-based medical devices not optimal for short- and medium-term applications [21]. Indeed, several works have reported that high molecular weight PLLA lasts from 2 to 5.6 years for its total resorption *in vivo* [23,24]. In this sense, different copolymerization approaches have been investigated to tune its degradability [6].

To date, the composite strategy has been mainly used for fabricating polymeric scaffolds with desired mechanical properties [25] For example, natural-polymer based composites have been reported to present an increased mechanical stability and improved cell interaction [26,27]. Overall, traditional polymer-based composites require large filler volume fractions to reach the desired physical and mechanical properties. On the contrary, nanocomposites usually offer improved physicomechanical performance at low loading fractions [28]. Therefore, an innovative approach could arise from the use of nanoparticles which would initiate hydrolytic degradation reactions of their hosting matrix and to thereby obtain scaffolds with tuneable degradation profiles. In a recent work, our group has shown that the hydrolytic degradation of PLLA could be markedly accelerated by adding zinc oxide nanoparticles (ZnO NPs) *via* H_2O dissociation on ZnO oxygen vacancy sites and the attack of this new hydroxyl groups to the PLLA ester bonds [29]. Thus, it could be hypothesized that the selective addition of ZnO nanoparticles into PLLA matrix would yield scaffolds with larger degradation rates. More interestingly, it has been reported that these nanoparticles preferentially kill high proliferative cells, like cancer cells, *versus* normal cells and are able, as well, to act as effective antibacterial agents [30]. Additionally, zinc oxide nanoparticles are economically feasible and have been approved by the American Food and Drug Administration (FDA) [31]. In one of our previous works we have demonstrated that when ZnO nanoparticles are loaded into PLLA they activate the material surface and drive cell differentiation at the same time that the release of zinc within the culture medium remains negligible [32]. Consequently, it could be expected that in addition to modulating the degradation kinetic of PLLA scaffolds, ZnO nanoparticles may as well stimulate a faster regeneration of the healing tissue. Additionally, it has been proven that the presence of zinc oxide avoids biofilm formation and bacterial colonization on the implant devices, hindering bacterial an eventual bacterial infection caused by the medical implant [33].

In this work we attempt to investigate the hydrolytic degradation mechanism of PLLA-based porous scaffolds that mimic the architecture of natural extracellular matrix (ECM). Since typically porosities of about 80–90% with a minimum average pore diameter of 60–100 μm are required for obtaining a successful cell penetration and vascularisation of the newly forming tissues [5], scaffolds having a pore diameter of 125–250 μm and porosities up to 90% have been fabricated by solvent casting/particulate leaching method (SCPL). The structural evolution of PLLA/ZnO scaffolds during their hydrolytic degradation in phosphate buffered saline (PBS) has been studied as a function of porosity and obtained results were compared to plain PLLA scaffolds. The pH changes induced by the release of the acidic by-products, the mass loss, the thermal transitions and the thermal stability of the scaffolds were monitored during the *in vitro* degradation. Furthermore, the morphological evolution of scaffolds upon degradation has been explored by scanning electron microscopy (SEM). The combination of the inherent properties of zinc oxide make ZnO NPs great candidates to develop cheap antibacterial scaffolds with tuneable hydrolytic profiles which are expected to aid cells during their proliferation process. Obtained results may be useful towards the development of antibacterial scaffolds that cover a wide range of degradation rates for potential tissue engineering applications.

2. Experimental part

2.1. Starting materials

PLLA with a number-average molecular weight (M_n) of 100.000 g/mol and a polydispersity index (M_w/M_n) of 1.85 has been supplied by Purac Biochem. Phosphate buffered saline (PBS) tablets and NaCl was supplied by Sigma Aldrich, chloroform (reagent ≥ 99.8%) was purchased from LabScan. Zinc oxide (ZnO) nanoparticles have been kindly purchased by L'Urederra Technological Centre (Spain).

3D scaffolds were prepared *via* solvent casting/particulate leaching method using chloroform as a solvent and NaCl particles (sieved to be in between 120 and 250 μm) as a porogen. Firstly, ZnO nanoparticles were homogeneously dispersed in chloroform *via* mild sonication (20% output for 1 min) in a Vibra-Cell™ CV 334 ultrasonic processor. These dispersed NPs were added to previously dissolved PLLA (in chloroform) to obtain a ZnO concentration of 1 wt% (with respect to polymer fraction). PLLA–ZnO dispersions were submitted to an additional sonication step for 5 min. Different amounts of NaCl particles were added them and the mixture was vigorously magnetically stirred for 1 h. The resulting materials were transferred to circular Petri-dishes and were dried until constant weight. Square-shaped (1×1 cm^2) scaffolds were punched out and placed in distilled water for 48 h at room temperature to leach out the salt particles. Non-porous samples were prepared in a hydraulic hot press by compression moulding at 200 °C for 4 min under a pressure of 150 MPa.

In vitro degradation of PLLA and PLLA/ZnO scaffolds with a porosity ranging from 0 to 90% was carried out in an oven at 60 °C. Samples with a surface area to volume ratio of 0.2 cm^{-1} were immersed in a PBS solution (PBS tablets dissolved in Milli-Q water; pH = 7.4) and they were removed after different periods of time. The pH evolution of the medium has been monitored using a 691 pH Meter (Metrohm).

Two samples from each composition and degradation time were removed from the PBS solution and weighed after wiping the surface with a filter paper to absorb the surface water. These samples were dried at 40 °C for 24 h and were weighed again (dry weight, W_d). Remaining weight (%RW) was then calculated:

$$\%\mathrm{RW} = \frac{W_d}{W_o} \cdot 100 \tag{1}$$

where W_o represents the initial weight of scaffolds (~30 mg).

2.2. Differential scanning calorimetry (DSC)

Thermal transitions of degraded scaffolds were determined using a Mettler Toledo DSC 822e calorimeter under nitrogen atmosphere (30 ml/min). Samples having 6 ± 1 mg were sealed in an aluminium pan and they were heated from 0 to 200 °C at a rate of 10 °C/min. The PLLA crystalline fraction X_c (%) has been determined as [34]:

$$X_c(\%) = \frac{\Delta H_f - \Delta H_c}{\Delta H_f{}^0} \cdot 100 \tag{2}$$

where ΔH_f and ΔH_c are respectively the enthalpy of fusion and cold crystallization of the samples determined on the DSC. $\Delta H_f^0 = 106$ J/g was taken as the heat of fusion of an infinitely thick PLLA crystal [35].

2.3. Thermogravimetric analysis (TGA)

Thermal stability of scaffolds was studied in a TGA METTLER TOLEDO 822e Thermal Gravimetric Analysis instrument by heating the samples from room temperature to 500 °C at 10 °C/min with nitrogen flux of 50 ml/min.

2.4. Morphological characterization

Scaffold morphology upon hydrolytic degradation has been analyzed in a Hitachi S-4800 field emission scanning electron microscope (FE-SEM) at an acceleration voltage of 5 kV. Surfaces were chromium-coated in a Quorum Q150T ES turbo-pumped sputter coater (5 nm thick coating).