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Polylactic acid organogel as versatile scaffolding technique

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ABSTRACT

Tissue engineering requires scaffolding techniques based on non-toxic processes that permits the fabrication of constructs with tailored properties. Here, a two-step methodology based on the gelation and precipitation of the poly(lactic) acid/ethyl lactate organogel system is presented. With this technique nanofibrous matrices that resemble natural extracellular matrix can be easily obtained, while allowing control over the mechanical properties of the device. Gelation temperature and the dynamics of the gelation of the organogel system are characterized, and the final mechanical and viscoelastic properties, as well as porosity, as function of the initial polymer concentration are described. We show that gelation temperature of the system is concentration independent and below 44.5 °C, which permits gelation at room temperature. Furthermore, mechanical properties are found in the range of the soft organic tissues, and the obtained micro-network architecture gives place to a flexible structure. Such structure presents tuneable elastic modulus and viscoelastic properties as function of nanofibers density. Moreover, centimetre-long tubular scaffolds with the diameter of medium-caliber blood vessels were produced. The fibrous nano-architecture mimics the native extracellular matrix fibres diameter and morphology was proven to be suitable to support endothelialization of the lumen of the tube. Thus, this strategy, based on biocompatible green compound might be promising for the fabrication of large 3D scaffolds for tissue engineering applications.

1. Introduction

Scaffolds play an important role on regenerative medicine owing to their capacity to direct tissue reconstruction by means of physical and chemical cues [1]. However, the scaffolds for tissue engineering on the market are still very limited [2]. Thus, in order to have a real impact on health society, it is very important to develop strategies and products that can be successfully translated toward the clinics. Current challenges for the translation include the development of processes that can control the scaffold physical properties in a tailored way (i.e., architecture, mechanical behaviour, porosity and diffusivity), while, at the same time, can be scaled.

The most used synthetic polymers for scaffold fabrication are the thermoplastic aliphatic polyesters like poly(lactic) acid (PLA), poly(glycolic) acid (PGA) and their co-polymers (PLGA). They have been applied on many biomedical therapies, whether in the form of active scaffold, carriers or holding structures, alone or in combination with other molecules, materials or cells [3,4]. Because of their thermoplastic and molecular properties, the techniques explored and used for their processing are numerous [5]. However, most of the known techniques use organic solvents to dissolve the polymer in order to be able to cast, mould or electrospun it, high temperatures to melt and extrude it. As a consequence, the presence of toxic residues or the degradation of the polymers due to heating, are main drawbacks. For instance, the presence of chloroform in casted scaffolds after one week drying can be up to 5000 ppm [6], while permitted level of residual chloroform for

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drugs in US is 60 ppm [7]. Another example is the degradation of the molecular chains that PLA suffers during the extrusion or any kind of process that uses heat, which, unless adequately considered during the design of the process, can compromise the final scaffold properties and mechanical behaviour [8,9].

Complexity on scaffold manufacturing increases when regulatory requirements are considered. Because cell based biomaterials are strongly regulated and are more costly to implement, acellular biomedical devices are normally preferred, when possible, on industry and clinics [10]. Therefore, such acellular devices have to act as guiding director of the regeneration process. Thus, properties like morphological microstructure and mechanical behaviour need to resemble the natural extracellular matrix of the target tissue, since these physic-chemical properties are known to affect the cell behaviour, growth and differentiation and therefore can be used to promote and direct tissue regeneration [11,12].

Mimicking the extracellular matrix requires structures on the nanometre range, and very few techniques are able to build them. Electrospinning, for instance, allows producing nanosized fibres. However, the obtained structures are usually non-woven and the creation of large, three-dimensional constructs, with controlled internal porosity, is limited. To overcome such limitations, is desirable to design alternative processes that can create three-dimensional nanofibrous interconnected porous matrices with good elastic properties and with clinically-relevant sizes. This is the case of the organogel phase separation process.

Organogels are defined as gels formed during the immobilization of an apolar phase (usually the solvent) within the spaces of a three-dimensional network formed due to the physical interactions amongst the self-assembled structures of compounds regarded as gelators (the polymer) [13]. These gels are characterized for being thermodynamically stable and having a thermoreversible gelation behaviour which depends on temperature, gelator concentration and gelator molecular structure. Moreover, organogels can present a series of different interesting characteristics like optical and viscoelastic properties, biocompatibility and the capability of being used as drug delivery agents [13,14].

Among organogels, some are composed by polyester/organic solvent systems. An example of this is the organogel obtained from the entrapment of the tetrahydrofuran (THF) inside a Polylactide (PLA) self-assembled network [15,16]. These PLA/THF solution systems can be gelated at low temperatures into a mould. Later, the three-dimensional gel can be phase separated by immersing the gel into water, which displaces the THF. During this exchange, the PLA network from the organogel precipitates due to a phase separation process and a fibrous three-dimensional structure is obtained. The organogel phase separation process permits to obtain highly porous scaffolds, with a microstructure that resembles the extracellular matrix, through a very simple and scalable process [16]. However, the use of toxic compounds, such as THF, poses toxicity concerns as well as risk of environmental harming.

The aim of this work was to develop a new organogel system based on the PLA and ethyl lactate (EtLac). EtLac is an FDA-approved compound, which is commonly used in food industry. It is a biodegradable, green solvent, whose degradation products can be metabolized by the human body [17]. For the PLA/EtLac pair, EtLac solvent is known to dissolve the polymer at 40–50 °C, and produce porous structures during its phase separation upon immersion into water [18]. In this work we describe that EtLac is able to allow the formation of a PLA organogel at room temperature, which can be used to produce three-dimensional shapes with control on the nanoarchitecture, porosity and mechanical properties.

This study characterizes a two-step process, based on the PLA/EtLac system, to obtain scaffolds for tissue engineering with high control on their properties and that does not harm the molecular integrity of the material. For such a purpose, the gelation temperature and the gelation dynamics were analysed as a function of polymer concentration. Their correlation with material shrinking upon gelation and, the mechanical and viscoelastic properties of the formed scaffolds, as well as their porosity and their diffusion capabilities were analysed. Moreover, a proof-of-concept of the biological activity of the fabricated scaffolds is presented.

2. Materials and methods

2.1. Materials

Poly−L/DL lactic acid 70/30 (Purasorb PLDL 70/38, inherent viscosity midpoint 3.8 dl/g, Mw ≈ 850,000 Da) (PLA) was purchased from Purac Biomaterials (The Netherlands).

(L)-Ethyl Lactate (EtLac) 99% photoresist grade was purchased from Sigma and used without further purification.

2.2. POLY(LACTIC) acid scaffolds preparation

Different amounts of PLA were dissolved in EtLac at 54 °C under stirring. For example, 7 g of PLA were dissolved in 100 mL of EtLac to obtain a 7% PLA solution (w/v). Subsequently, the PLA/EtLac solution was poured into the desired mould and placed at cold temperatures until complete gelation occurred. When the gel was completely formed, it was removed from the mould and immersed into milliQ water. Finally, due to the diffusion of EtLac into water, solvent was exchanged and PLA precipitated by phase separation. The scaffolds were washed several times with water, to remove residual EtLac.

For long-term storage, PLA scaffolds were lyophilized and kept in dry conditions. Before usage, samples were reconstituted in water.

2.3. Gelation process characterization

Gelation was characterized by rheology, with a DHR rheometer (TA Instruments) with a Peltier Plate as a temperature control system.

2.3.1. Viscosity

PLA/EtLac solutions of 4%, 7% and 10% concentrations were heated until 70 °C and studied in flow mode. Viscosity was measured for a range of shear rates going from 10^{-5} till 10^{3} s^{-1} ($n = 3$).

2.3.2. Gel temperature

The Gel Temperature (T_{gel}) was found following a methodology based on the gelator concentration independency principle [19]. For this purpose, the gelation time as a function of temperature was studied for the different concentrations. Obtained gelation curves were plotted, and the convergence point was found by extrapolation. The gelation times (t_{gel}) needed for the creation of the gelation curves were found by analysing the crossing of the tanδ during the gelation process for each sample at each temperature in a tanδ versus time plot [20]. Studied concentrations ranged from 4% until 8% and studied temperatures ranged from 5 °C until 25 °C. The characterization of the tanδ evolution was carried out in oscillatory mode conditions. Frequencies used for the assay were ranged between 0.5 and 2 Hz and the applied strain was 0.5% ($n = 4$).

2.3.3. Gelation kinetics

Kinetics was studied in oscillatory conditions, using frequencies ranging between 0.5 and 2 Hz and a 0.5% strain. The development