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Research Paper

Characterization, mechanical behavior and *in vitro* evaluation of a melt-drawn scaffold for esophageal tissue engineering

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ABSTRACT

Tubular esophageal scaffolds with fiber diameter ranging from 13.9±1.7 μm to 65.7±6.2 μm were fabricated from the highly elastic poly(L-lactide-co-ε-caprolactone) (PLC) *via* a melt-drawing method. The morphology, crystallinity, thermal and mechanical properties of the PLC fibers were investigated. They were highly aligned and have a uniform diameter. PLC is found to be semicrystalline consisting of α- and β- lactide (LA) crystals. The crystallinity increases up to 16.8% with increasing melt-drawing speeds due to strain-induced crystallization. Modulus and strength increases while ductility decreases with an increase in crystallinity of the PLC samples. Moisture will not degrade the overall tensile properties but affect its tangent modulus at the low strain. L929 cells are able to attach and proliferate on the scaffolds very well. The cells seeded on the scaffolds show normal morphology with >90% cell viability after 6 days of culture. These results demonstrate that the PLC fibrous scaffold has good potential for use in esophageal tissue engineering application.

1. Introduction

Esophagectomy is the primary curative therapy for the patients with resectable esophageal carcinoma (Pennathur et al., 2010). Preferably, the digestive continuity after esophagectomy could be restored with the esophageal replacement by tissue engineering (TE) techniques. It would potentially offer the ready-to-use replacement with structural integrity and normal function (Chian et al., 2015). There are four main technologies that are commonly utilized in esophageal TE, namely the extracellular matrix (ECM) decellularization (Sjöqvist et al., 2014), the fibrous scaffold fabrication (Leong et al., 2009), the molding of scaffold (Lim et al., 2010), and the cell sheet technology (Ohki et al., 2014). Nonetheless, problems, *e.g.* stenosis and leakage, frequently occur when full-thickness circumferential esophageal replacement was

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performed (Tan et al., 2012). Most of the reported approaches did not emphasize on the patient-specific scaffolds that could match both the biomechanical properties (Rao et al., 2003) and the physical dimensions (Bouma et al., 2000) to the host esophagus. These two criteria are very important for the temporary circumferential construct. The difference in physical dimensions of the scaffold to the remaining native organ can lead to blockage or accumulation of food bolus during its transportation down. Moreover, different patients have different sizes of esophagi (Bouma et al., 2000). In addition, biomechanical properties of the scaffold are also extremely important as the esophageal scaffold is a temporary construct that will be exerted to various stresses and strains during food bolus transportation. Hence the scaffold should be mechanically compatible with the native organ so as to facilitate food bolus transportation before the growth and recovery of esophageal tissues.

The selection of materials for TE depends on the mechanical properties needed for the implantation and the desired degradation time frame (Middleton and Tipton, 2000; Leong et al., 2008). Some biomaterials that have been employed in esophageal TE usually are the natural materials e.g. collagen (Natsumi et al., 1990) and chitosan (Lim et al., 2010); and the synthetic materials e.g. polylactide (PLA) (Leong et al., 2009), poly(ε-caprolactone) (PCL) (Diemer et al., 2014), and poly (glycolic acid) (PGA) (Sato et al., 1994). In particular, poly(L-lactide) (PLLA, a form of PLA) and PCL were intensively studied in TE. Both of them are linear aliphatic polyesters with similar chemical structures. As compared to PCL, PLLA has more ester groups per chain, hence it degrades more readily by hydrolysis and shows faster biodegradability (Nampoothiri et al., 2010). With a crystallinity of ~37% (Middleton and Tipton, 2000), PLLA has superior mechanical properties such as high stiffness and high strength. However, PLLA is brittle at body temperature of 37 °C due to its high glass transition temperature (T_g) of 60–65 °C. Therefore, it is not suitable to be used for making the esophageal scaffolds as failure might occur during food bolus transportation *in vivo*. On the contrary, PCL has more alkyl units between the ester links, which allows it has more flexible chains. It also has a low T_g (~ −60 °C), thus it behaves as a tough polymer at 37 °C. Copolymers of PLLA and PCL, i.e. poly(L-lactide-co-ε-caprolactone) (PLC), had been developed in order to take advantage of the improved synergistic properties offered by both polymers (Peponi et al., 2012). Some of these copolymers exhibits a rubber-like elasticity and has shape memory properties (Peponi et al., 2013). In esophageal TE, PLC has been electrospun to mimic epithelium (Zhu et al., 2007). PLC with L-lactide (LA): ε-caprolactone (CL) molar ratio of 70:30 was used in our previous paper (Tan et al., 2015) for esophageal TE and it was shown to have tensile properties comparable to the native esophagus. It was also reported that PLC with a higher LA content were less crystalline and had comparatively high water absorption, which would result in a higher hydrolytic degradation rate (Garkhal et al., 2007). A sufficiently high degradation rate after tissue regeneration will be preferred in TE because foreign materials carry a risk of inflammation (Langer, 1994).

Melt spinning (Kase and Matsuo, 1965) and melt electrospinning (Larrondo and Manley, 1981) are the two common methods to produce microfibers from polymer melt. The former uses an extrusion force and the latter employs electric charge to form polymer fibers, whereby the fibers were then drawn on rotating mandrels to steer the fiber orientation. The fiber drawing process provides fiber elongation and it decreases the fiber diameter while enhances its strength (Wong et al., 2008). Melt-drawing, which is a simplified version of these polymer melt microfibers processing was previously built in-house in the laboratory (An et al., 2012). Melt-drawing offers a number of advantages over other TE techniques. One of these advantages is the topography control, which can be customized to achieve the required mechanical properties of the scaffolds. Melt-drawing has the capability to produce highly aligned microfibers with a simple set up. Scaffolds with highly aligned fibers were shown to speed up the secretion of ECM by the cells when compared to randomly deposited fibers (Lee et al., 2005). These ECM can then provide mechanical support to tissues and act as a substrate for cell adhesion and differentiation (Chiquet et al., 1996). Another distinct benefit of using this technique is that no undesirable toxic solvent is used.

Microfibrous scaffolds were fabricated by melt-drawing PLC for esophageal TE in this work in which the fabrication technique shows a great potential in producing the patient-customized esophageal scaffolds. PLC is a semicrystalline polymer, thus the scaffolds' mechanical properties (Kanaga Karuppiah et al., 2008) and biodegradation (Han et al., 2010) will highly rely on their fibers' morphology and crystal structure. Meanwhile, the morphology and microstructure of melt-drawn PLC must be varied in terms of different processing parameters, particularly the melt-drawing speed. The aim of this work is to fabricate the highly aligned PLC microfibers by melt-drawing and then systematically study the influences of melt-drawing speeds on their morphology, crystallinity, thermal properties, tensile properties and *in vitro* biocompatibility. The crystalline structure of the PLC was examined in detail before and after melt-drawing, which enables us to achieve an in-depth understanding of structural evolution during the fabrication of this microfibrous scaffold.

2. Experimental

2.1. Fabrication of PLC tubular scaffold

PLC statistical copolymer (Corbion Purac, PURASORB® PLC 7015) was used without further treatment or purification. A customize-made table-top microfiber melt-drawing device was employed to fabricate scaffolds (Tan et al., 2015; An et al., 2012). The process involves melting the PLC in a melt holder at ~150 °C, and then continuous pulling a single microfiber from the melt. It was first manually initiated with a needle inserted into the melt through the orifice at the bottom of melt holder, where the microfiber was pulled down from the melt pool. The microfiber was then being continually collected on a rotating cylindrical mandrel as illustrated in Fig. 1. The mass flow rate of PLC in the melt-drawing process was ~0.2 g/min when using a melt-holder orifice diameter, d of 2 mm.