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SOLVENT-FREE MELT-DRAWING OF ALIGNED POLY(L-LACTIDE-CO-E-CAPROLACTONE) MICROFIBRES INTO TUBULAR SCAFFOLD FOR ESOPHAGEAL TISSUE ENGINEERING

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ABSTRACT: A solvent-free melt-drawing of aligned poly(L-lactide-co- ϵ -caprolactone) copolymer (PLC) microfibers into the tubular scaffold with the endocircular and exolongitudinal patterns has been investigated for the esophageal tissue engineering. The PLC microfibrinous tubular scaffold was melt-drawn onto the 3D printed grooved mandrel. The experimental results show that the parallel grooves normal to the microfiber direction formed on the inner surface of the tubular scaffold. The surface topography of the tubular scaffold will mimic the endocircular and exolongitudinal muscle layers of the esophagus when the scaffold is turned inside out. It is proposed that this surface architecture may induce the cells orientation and cells attachment during its interaction with the individual smooth muscle cell. A large shrinkage of scaffold in dimension is observed along the fiber direction after the removal of scaffold from mandrel, which indicates that the PLC scaffold has a high elasticity. Therefore, the PLC scaffold will be mechanically compatible with the esophagus. Furthermore, it is suggested that the melt-drawing could be combined with the bioprinting technologies to print the tubular organs efficiently.

INTRODUCTION

The esophageal cancer causes an estimated 400,000 deaths annually. It is the sixth leading cause of the cancer-related deaths worldwide (Smith et al. 2008). The American Cancer Society estimates that ~17990 new esophageal cancer cases will be diagnosed and ~15,210 deaths would result from the esophageal cancer in United States in 2013. Moreover, the esophageal cancer is much more common in some other countries or regions, such as Iran, northern China, India, southern Africa and so on (American Cancer Society 2013).

The esophageal cancer could be treated by the surgical removal of a large portion of the affected esophagus, i.e. esophagectomy (American Cancer Society 2013). Currently, the patients' stomach, jejunum, or colon are frequently used for the reconstruction of esophagus. However, patients may suffer from the postoperative malnutrition due to the poor functioning of the substitute and the cut of original gastrointestinal tract (Fuchs et al. 2001). In addition to the organ transplantation, artificial stents have been employed in the esophageal replacement including the silicone, Nitinol, stainless steel, polyurethane, etc. Nevertheless, the gastroesophageal reflux disease, the recurrence of strictures, and the stent migration frequently occurred after the stent replacement (Hindy et al. 2012).

The tissue engineering of esophagus could be advantageous for the gap replacement after esophagectomy due to its exact replacement of the functioning tissue structure. In addition, no prosthetic materials or harvested gastrointestinal tract is needed from the patient. Esophagus is a hollow and highly distensible muscular tube for the food bolus transportation from mouth to stomach in vertebrates via peristalsis (Strandring et al. 2005). The organization of tissues within the esophageal wall from lumen outwards is in the following sequence, namely the mucosa, the submucosa, the muscularis externa, and the adventitia (Strandring et al. 2005). Since esophagus is an elastically compliant organ, the esophageal tissue engineering requires the mechanically compatible biomaterials (Ritchie et al. 2006). In the past decades, many efforts have been made in the esophageal tissue engineering (Ike et al. 1989; Miki et al. 1999; Badylak et al. 2005; Ritchie et al. 2006; Saxena et al. 2009). However, few literatures are involved in the growth of directional smooth muscle tissue that results in the formation of the endocircular and exolongitudinal bilayer muscle layers in the muscularis externa. The two muscle layers play the main role in the esophagus peristalsis. The main functioning muscle cell found in this bilayer muscle tissue is the smooth muscle cell (SMC). In order to grow the bilayer tissues in the preferred directions, the SMC arrangement with controlled scaffold geometry is of great importance. For instance, the orientation and attachment of cardiac myocyte were found to be significantly affected by the surface topography of its substrate (Deutsch et al. 2000). In this work, it is thus proposed to produce an elastically compliant tube as the esophagus scaffold with the topography that mimics the endocircular and exolongitudinal muscle layers.

EXPERIMENTAL

Model

The SMC is a mononucleus cell with the spindle-like morphology. It is the main functioning cell in the endocircular and exolongitudinal bilayer muscle layers in the muscularis externa (Gong et al. 2013). Unlike in 2D models, cells in 3D matrix extend pseudopodia following matrix fibrils (Li

et al. 2003). The SMCs cultured on scaffolds with microchannels were reported to be uniformly aligned along the microchannel. These SMCs were found to have an increased expression of smooth muscle α -actin, indicating that these regularly aligned cells are shifting from the synthetic phenotype to the contractile phenotype (Shen et al. 2006). Hence, an ideal tubular scaffold, as shown in Figure 1, is proposed to resemble the surface topography of the esophageal muscularis externa. The scaffold with the surface topography closely mimicking the bilayer muscle layers may be able to regenerate a functioning esophagus.

Figure 1. Schematic illustration of esophageal scaffold design

Materials and Methods

The microfiber melt-drawing device was previously built in our lab (An et al. 2012). It consists of five sub-systems: the melt holder, the positioning and motion system, the heating system, the collecting system (a mandrel) and the framework.

Elastomeric PLC with LLA:CL ratio of 70:30 was polymerized. PLC was melted in the melt holder at 150°C. Microfiber was pulled from a pool of PLC melt in a direct and straight manner as described in (An et al. 2012). It was then collected on a rotating cylindrical mandrel by a continuous drawing of the single microfiber from the melt. The rotation speed of the mandrel was 500rpm. The melt holder moved to-and-fro in the translational motion in order to produce the tubular scaffold. In this work, a mandrel with grooves was designed and then 3D printed. It was used to melt-draw the PLC microfibers.

Figure 2. Schematic illustration showing the shrinkage measurement of fibers after cutting from mandrel

After the melt-drawing process, the tubular scaffold was removed from the cylindrical mandrel. Preliminary morphological examination of the scaffold was carried out by means of an optical microscope (Olympus CKX41) after its shrinkage. For the shrinkage analysis of the fibers, as exhibited in Figure 2, the microfibrinous scaffold was cut from the cylindrical mandrel in the longitudinal direction. The shrinkage in the fiber direction was measured with the removal time of scaffold. Original fiber length ($t=0$) was obtained from the mandrel diameter when assuming that the thickness of the scaffold is negligible. A graph was plotted to show relationship between the fiber shrinkage and the removal time of scaffold from the mandrel.

RESULTS AND DISCUSSION

Morphology

Figure 3(a) shows the melt-drawn PLC tubular scaffold generated from the cylindrical mandrel. The circumference of the PLC tubular scaffold shrinks when it is removed from the mandrel. The aligned microfibers can be clearly seen in Figure 3(b).

Figure 3. (a) Macrograph of the melt-drawn PLC tubular scaffold generated from the cylindrical mandrel and (b) optical micrograph of the microstructure of the melt-drawn PLC scaffold

The 3D printed mandrel with parallel grooves is shown in Figure 4(a). Microfibers were drawn onto the mandrel. In order to observe the forming grooves, the scaffold was longitudinally cut as illustrated in Figure 4(b).

Figure 4. Macrograph of (a) melt-drawn PLC on the grooved mandrel and (b) parallel grooves formed on the inner side of the scaffold using the 3D printed grooved mandrel

It is clearly seen that the parallel grooves have formed in the perpendicular direction of the fibers. The tubular scaffold has the exolongitudinal grooves with endocircular microfibers when it is turned inside out. It is suggested that this surface geography might be capable of resembling the surface topography of the endocircular and exolongitudinal muscle layers for esophagus when it is turned inside out. Essentially, the arrangement of the SMCs in a controlled architecture could results in cells orientation and cells attachment. Consequently, muscle tissues could be grown in the preferred directions.

Shrinkage Analysis

After the removal from the mandrel, the tubular scaffold was found to shrink at a very fast rate initially, followed by a sluggish shrinkage over a few hours. To investigate the shrinkage rate, the tubular scaffold was longitudinally cut into a flat sheet. The length of the fibers sheet was measured at a different time after it is being removed.

Figure 5. Plot of fiber length (mm) vs. time (min) after the tubular scaffold being cut from mandrel

Figure 5 shows a rapid decrease in the fiber length of the PLC scaffold once it is cut from the mandrel. After a sharp drop of fiber length during the first minute, the fiber length starts to decrease slowly in the following 9 minutes. Shrinkage becomes very sluggish after 10 minutes and basically terminates after 120 minutes. It is speculated that PLC is susceptible to high tension when they are melt-drawn onto the mandrel due to their high elasticity. As a result, the PLC microfibrinous tube will shrink rapidly as the stress is suddenly removed once the tube is removed from the mandrel. The residual stresses that remain in the PLC microfibers would gradually release after the sudden removal of stress, which results in the following sluggish shrinkage in the tube.

Melt-Drawing and Bioprinting

One of the most exciting developments in tissue engineering is the bioprinting (Mironov et al. 2006; Mironov et al. 2007; Jakab et al. 2010). The bioprinting technique prints cells and usually employs hydrogels as the scaffolds. In spite of the fact that the tissue-engineered esophagus must

be elastically complaint, only hydrogels might not be able to provide the sufficient strength. Besides, SMCs in 3D hydrogel matrix had less stress fibers, less focal adhesions and cell spreading (Li et al. 2003). It will be an ideal solution that solid scaffolds are involved in the esophagus bioprinting. The conventional solid scaffold fabrication techniques include the solvent casting, the gas foaming, the freeze drying, the particulate leaching, the electrospinning and the phase separation. However, these methods often use the organic solvents during processing, which is not cell-friendly. Moreover, they are mostly not automated. Melt-drawing is a possible way combining the solid scaffolds in bioprinting because it is solvent-free. In addition, it is half-automated, which only requires an initial pull of microfiber from the polymer melt as described previously. In the meanwhile, the combination of melt-drawing and bioprinting is not only beneficial for the esophageal tissue engineering, but also for all the tubular organs such as trachea, blood vessels and small intestines. The utilized mandrel may have different patterns to create the preferred surface topography on the inner surface of the tubular scaffold during the melt-drawing.

CONCLUSIONS

A tubular microfibrinous scaffold with grooves, aiming to regenerate the endocircular and exolongitudinal bilayer muscle tissues of the normal esophagus, was designed and fabricated. A solvent-free melt-drawing of aligned PLC microfibers onto the 3D printed grooved mandrel was utilized in the fabrication. The parallel grooves formed in the normal direction of microfibers on the inner surface of the tubular scaffold. Hence, it is suggested that the surface topography of the melt-drawn tubular scaffold could mimic the endocircular and exolongitudinal muscle layers of the esophagus when the scaffold is turned inside out. This surface topography may induce the cells orientation and cells attachment during its interaction with each SMC. A large shrinkage of the scaffold in circumference along the fiber direction is immediately observed after the removal of scaffold from the mandrel, which indicates that the PLC scaffold has a high elasticity. Hence, the PLC tubular scaffold is mechanically compatible with the esophagus. These results suggest that this 3D micropatterned biodegradable microfibrinous scaffold may be useful for guiding SMCs to grow into the functioning esophagus. It is proposed that the melt-drawing could be combined with the bioprinting technologies to print the esophagus with the solid scaffolds to provide the required mechanical strength and facilitate the directional growth of cells.

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