In Situ Gelatinization Manufacturing of Fibrin Blood Vessel Scaffolds

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Introduction: For a tissue-engineered construct to grow beyond 100-200μm (the diffusion limit of oxygen), new blood-vessel formation is required[1]. As product of natural coagulation process and reconstruction matrix of injured blood vessel, fibrin is a promising scaffold material for constructing tissue engineered blood vessels (TEBV)[2]. In this study, fibrin hydrogel scaffolds were fabricated by the newly proposed rapid prototyping process called *in situ* gelatinization manufacturing (ISGM). The micro morphology of the scaffolds was analyzed. A TEBV model with double cell layers was generated and evaluated using this technique.

Methods: (1) Scaffold fabrication and characterization. The ISGM system was developed by Center of Laser Rapid Forming, Tsinghua University. Fibrinogen (15%) and gelatin (3%) solution mixture (1:1, v/v) and thrombin solution (10U/ml) were prepared [Fig.1(a)] and put into two material feeding injectors of a coaxial-extrusion nozzle respectively, then the materials were extruded, mixed at the nozzle outlet and stacked up layer-by-layer into a low temperature chamber (~4℃) under the drive of a digital model developed in computer [Fig.1(b)]. Low-temperature scaffolds with pre-designed structures were fabricated, which were supported by instantly gelled gelatin and partially transformed fibrin from fibrinogen by thrombin. After immersed in 37℃ water or medium for 30min for fibrin curing and gelatin leaching, fibrinogen in the scaffolds was totally transformed into insoluble water-swollen fibrin while most of the gelatin in the scaffolds dissolved in water [Fig.1(c)]. For micro morphology analysis, some scaffolds were freeze-dried and evaluated with Scanning Electron Microscope (SEM).

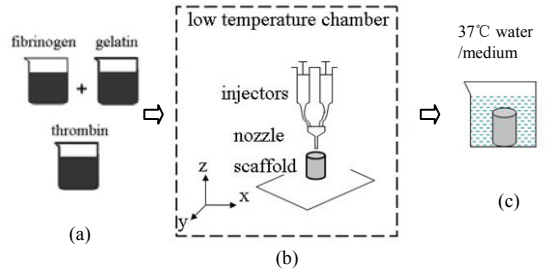

Figure 1. The ISGM process, (a) material preparation, (b) low-temperature scaffolds fabrication, (c) process of fibrin curing and gelatin leaching.

(2) TEBV model constructing and evaluation. A tubular smooth muscle cell (SMC) construct was fabricated via an improved ISGM process by mixing SMC suspension (4×10^6/ml) into fibrinogen/gelatin mixture in the material preparation process. Human Umbilical Vein Endothelial Cell (HUVEC) suspension (1×10^5/ml) was then injected into the tube [Fig.2(a)]. After 5 hour process for cell adhering [fig.2(b)], culture medium was added into the dish [Fig.2(c)]. After 1 week cultivation, the construct was fixed for histological analysis.

Results: Fig. 3 shows the fabricated fibrin scaffolds via ISGM process. The scaffold had micro fibrous morphology of fibrin and a little membrane structure of gelatin residue (Fig. 4). The fiber diameter was less than 1μm. In the constructed TEBV model, abundant SMCs survived in the micro fibrous structures after one week cultivation [Fig. 5(a)]. Fig. 5(b) shows that HEBVCs adhered, spread on the tubular lumen and formed an endothelial cell monolayer.

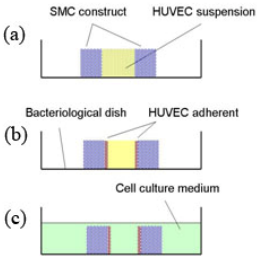

Figure 2. Constructing TEBV model with double cell layers.

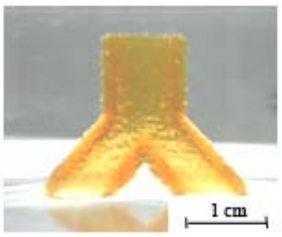

Figure 3. Fibrin scaffold fabricated via ISGM

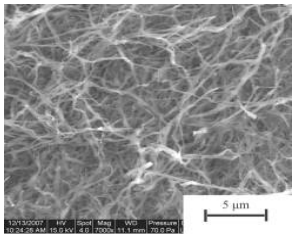

Figure 4. Micro morphology of fibrin scaffold

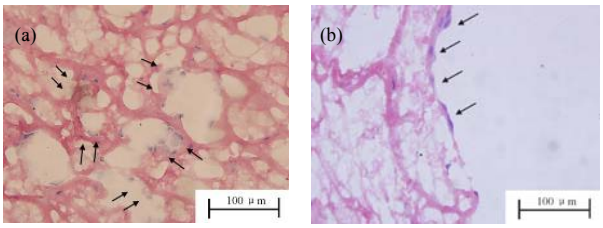

Figure 5. HE staining images of TEBV model after 1 week cultivation. (a)SMCs in the fibrin construct, (b) HUVECs lining on the construct lumen surface

Conclusions: ISGM process can make stable fibrin blood vessel scaffolds by an accurately controlled manner of layer-by-layer stacking. The scaffolds have micro fibrous morphology that is suitable for cell attachment and growth. Fibrin constructs containing living cells can also be fabricated by this ISGM system. The scaffolds have good compatibility with SMCs and endothelial cells. By further developing the ISGM system into a multi-nozzle system, tissue engineering constructs composed of multi-materials and contained two or more cell types were also fabricated, which was discussed elsewhere[3]. This process is a promising approach for engineering organs with build-in vascular networks.

Reference: 1. Rouwkema J, et al. Trends Biotechnol. 2008; 26:434-441. 2. Jockenhoevel S, et al. Eur J Cardiothorac Surg. 2001; 19:424-430. 3. Li S, et al. J Bioact Compat Polym. 2009, 24(3):249-265.