Polymer-Based "Glue Scaffold" with Nanometer-Sized Extracellular Matrix Films for Tissue Engineering

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Statement of Purpose: In tissue engineering, synthetic polymer for fabrication of scaffolds to seed cells is an attractive material motif due to easy manipulations of its 3D structure or mechanical properties. To support adhesion of cells, certain polymer-based scaffolds, require coating of the surface with bioactive molecules. However, the coating tends to form with uncontrolled thickness, which then alters surface structure [1] and physical properties of the scaffolds [2]. Thus, the goal of the present study is to explore a novel approach for coating a polymer-based tissue engineering scaffold to enhance cell adhesion. We focused on layer-by-layer (LBL) film of fibronectin (FN) and gelatin (FN-G film) [3] to fabricate thickness-controllable cell-adhesive extracellular matrix (ECM) film on polymer scaffolds. As a proof of concept work, we choose electrospun fibrous poly(carbonate urethane)urea (PCUU) [4] as a scaffold.

Methods: For preparation of FN-G films, PCUU scaffolds (thickness: 0.1~0.2 mm) were immersed in 0.04 mg/ml fibronectin (Mw = 4.6×10^5, Sigma-Aldrich) and 0.04 mg/ml gelatin in 50 mM Tris-HCl (pH = 7.4) for 2 min each. After each immersion step, the PCUU was washed with 50 mM Tris-HCl (pH = 7.4) for 1 min. After nine or twenty-one alternating steps of immersion in fibronectin and gelatin, $(FN/G)_4FN$ or $(FN/G)_{10}FN$ films were formed onto the scaffold. For visualization of fibronectin and gelatin in fluorescence microscopy, rhodamine-labeled fibronectin (Rh-FN; Mw = 2.5×10^5, Cytoskelton) was obtained from a commercial source and gelatin was labeled with fluorescein isothiocyanate (FITC) as reported previously. Bladder smooth muscle cells (BSMC, 1×10^4 cells) or UROtsa cells (4×10^5 cells) were seeded on PCUU (3.5 mm × 3.5 mm) with or without FN-G film resting at the bottom of cell culture inserts and cultured under standard conditions for up to 2 days.

Results: To confirm the adsorption of FN and gelatin on PCUU, FN-G films were prepared using rhodamine-labeled fibronectin (Rh-FN) and FITC-labeled gelatin (FITC-G). A confocal laser microscopy (CLMS) of fluorescent $PCUU^{FN-G}$ revealed fibrous PCUU network with Rh-FN and FITC-G. We estimated the amount of the ECM film by quantifying the fluorescence intensities of Rh-FN and FITC-G after each step of the LBL process. When we prepared FN-G films by alternate immersion of PCUU in Rh-FN and gelatin without fluorescence labeling, a stepwise increase of red fluorescence was observed. Similarly, in preparation of FN-G films with non-fluorescent fibronectin and FITC-G, green fluorescence increased in a stepwise manner. However, when a PCUU scaffold was immersed in a solution of Rh-FN repeatedly but not in gelatin, the fluorescence intensity stayed low and constant after two immersions. This implies that the lack of gelatin caused the saturation of Rh-FN on the surface of PCUU scaffold. By using scanning electron microscopy (SEM), we confirmed that surface structure of PCUU hardly change through the preparation of FN-G film. These results demonstrated successful preparations of thickness-controlled FN-G films on PCUU scaffolds with unmodified 3D structure. When we cultured BSMCs on FN-G film–coated PCUU scaffold ($PCUU^{FN-G}$), many BSMCs exhibited spread cell morphology, demonstrating that the FN-G film provided support for cell adhesion onto the scaffold. Since the bladder wall tissue in vivo is composed of transitional (superficial, intermediate, and basal) urothelial cells (UCs) lining the smooth muscle layer [5], fabrication of multilayered UCs on top of BSMC-seeded $PCUU^{FN-G}$ should yield a more realistic structure for engineered bladder tissues. For this purpose, we tried to fabricate multilayered UROtsa cells by culturing large amount of UROtsa cells on $PCUU^{FN-G}$. H&E stained frozen sections revealed that 2~3 layered UROtsa cells were successfully fabricated on $PCUU^{FN-G}$.

Conclusions: We present fibrous PCUU scaffold coated with nanometer-sized film of fibronectin and gelatin by LBL technique as "glue scaffold". The enhanced cell adhesion and survival of BSMCs and multilayered UROtsa cells on $PCUU^{FN-G}$ achieved in the present work indicate that this novel approach for coating of scaffolds is a promising improvement for engineering of bladder tissues in vitro. Because FN-G film formation is based on non-specific physical adsorption of fibronectin onto polymer as an initiating layer and its subsequent molecular interactions with gelatin, this technique may be applicable to other polymer-based scaffold systems to benefit various tissue engineering applications.

References: [1] Chen G. **et al.** Macromol Biosci, 2002. [2] Yunos DM. et al. J Mater Sci 2008. [3] Matsusaki M. Adv Mater, 2012. [4] Hong Y. et al. Biomaterials, 2010. [5] Orabi H. et al. Sci World J, 2013.