A Gelatin Polyurethane Composite Electrospun Scaffold with Cardiac Tissue-Compliant Character

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Statement of purpose: Tissue compliant scaffolds are preferred in cardiac tissue engineering as they promote the contractility and maturity of human pluripotent stem cell-derived cardiomyocytes (hPSC-CM) [1]. Degradable polar/hydrophobic/ionic polyurethane (D-PHI) is an established in-house immunomodulatory polyurethane [2]. Previously, an electrospun elastomeric polyurethane composite of D-PHI and polycarbonate polyurethane (PCNU) showed good compatibility with A10 vascular smooth muscle cells and triggered minimal foreign body response in a rat subcutaneous implant study [3]. However, the modulus of this scaffold remained too elevated, and took too long to degrade for cardiac tissue engineering applications [3]. Gelatin is often blended with synthetic polymers to change moduli and to improve hydrophilicity and cell attachment [4]. This study characterized a cardiac tissue-compliant and fast-degrading gelatin/D-PHI/PCNU composite scaffold produced via double-spinneret co-electrospinning.

Methods: D-PHI with a light-activated free radical initiator and PCNU were prepared using existing protocols [3]. A 30% (weight) 50:50 D-PHI/PCNU-hexafluoroisopropanol (HFIP) solution and a 7% 80:20 gelatin/PCNU-HIFP solution were electrospun simultaneously onto a single collecting mandrel in a custom-built Inovenso Double-nanospinner. The voltages for the D-PHI/PCNU spinneret, collecting mandrel, and gelatin/PCNU spinneret were +18 kV, -6 kV, and +20 kV, respectively. The flow rates for the D-PHI/PCNU and gelatin/PCNU spinnerets were 0.5 mL/s and 1.875 mL/s. D-PHI/PCNU fibres were cured in-flight by UV. The electrospun scaffold was then crosslinked in 50 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) ethanol solution for 24. The fibre morphology was imaged using scanning electron microscopy (SEM; Hitachi FlexSEM 1000) and analyzed using ImageJ (version 1.53a); three random regions of interest (area: 25.6 µm by 19.2 µm) were scanned. To assess cell viability and adhesion, hiPSC-CMs were cultured on laminin-coated gelatin/D-PHI/PCNU scaffolds for 7 days and then analyzed using live/dead staining and immunostaining against sarcomere actinin and connexin 43 (Cx43). Scaffold degradation over time was quantified by measuring weight loss in 0.01 U/ml [5] collagenase or 10 U/ml [6] cholesterol esterase (CE) PBS solutions at 37 °C (both macrophage related enzymes). The tensile elastic moduli of the as-made and degraded scaffolds in the fibre direction were measured at 5% strain (CellScale).

Results: The morphology of the gelatin/D-PHI/PCNU is shown in **Figure 1A**. hiPSC-CMs adhered well and showed 95%+ viability on the scaffold after 7 days (**Figure 1B**). The cells also showed aligned and organized sarcomeres, and border-localized Cx43 (**Figure 1C-D**). In collagenase, the scaffold lost nearly 50% of its weight after 1 day due to the loss of gelatin and continued to slowly degrade by hydrolysis (**Figure 2A**). In the CE solution, the gelatin-scaffold degraded steadily and had a 90% weight loss after 28 days (**Figure 2B**). The elastic modulus of the as-made scaffold was 300 kPa in the polyurethane fibre direction (**Figure 3**), which falls near the post-diastolic modulus range of cardiac tissue (200-500 kPa [7]). The modulus dropped to about 50 kPa (**Figure 3**) after 28 days of degradation in either enzyme solution, which is close to the pre-diastolic modulus (10 kPa [7]).

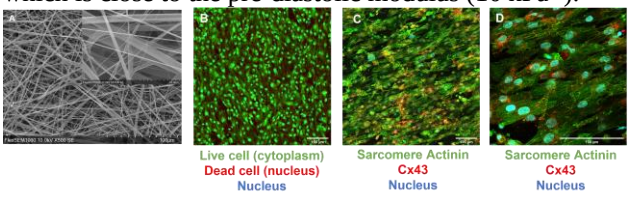

Figure 1: **A**: SEM image of the gelatin/D-PHI/PCNU scaffold. **B**) Live/dead, immunostaining images with **C**) 20X and **D**) 60X magnification of hiPSC-CMs cultured on the gelatin/D-PHI/PCNU scaffold after 7 days.

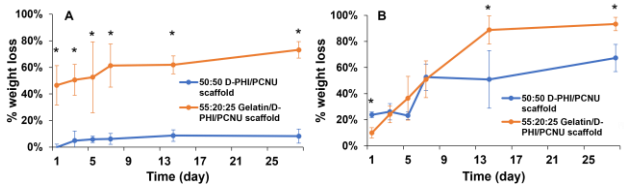

Figure 2: Weight loss of the gelatin/D-PHI/PCNU scaffold and D-PHI/PCNU scaffold in collagenase (**A**) and CE solution (**B**). *: p<0.05 compared to the D-PHI/PCNU scaffold.

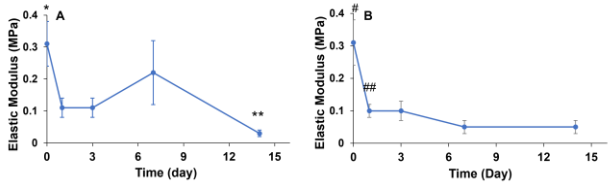

Figure 3: Elastic modulus of as-made and degraded gelatin/D-PHI/PCNU scaffold with collagenase (**A**) and CE (**B**). *: p<0.05 compared to Day 1, 3 and 14. **: p<0.05 compared to the rest. #: p<0.05 compared to the rest. ##: p<0.05 compared to Day 7 and 14.

Discussion/Conclusion: This study showed that a gelatin/D-PHI/PCNU composite scaffold is cardiac tissue compliant, enables healthy hiPSC-CMs growth, and degrades within a month to physiologically relevant moduli values in the presence of tissue relevant enzymes.

Reference

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