Fabrication of Dual-Functional Electrospun Nanofiber Scaffolds for Liver Tissue Engineering

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Introduction:

We have designed a novel dual-functional electrospun nanofiber scaffold through the layering of two nanofiber meshes that are modified differently to induce two separate biological responses from hepatocytes (Figure 1). The first nanofiber layer is galactosylated to mediate hepatocyte attachment and aggregation formation, while the second layer is loaded with 3-methylcholanthrene (3Mc) to enhance cytochrome P450 activity of hepatocytes. Primary rat hepatocytes cultured on the galactosylated nanofiber scaffolds loaded with different concentrations of 3Mc will be compared for their cell attachment efficiency as well as their P450-dependent 7-ethoxycoumarin O-deethylase (ECOD) activity.

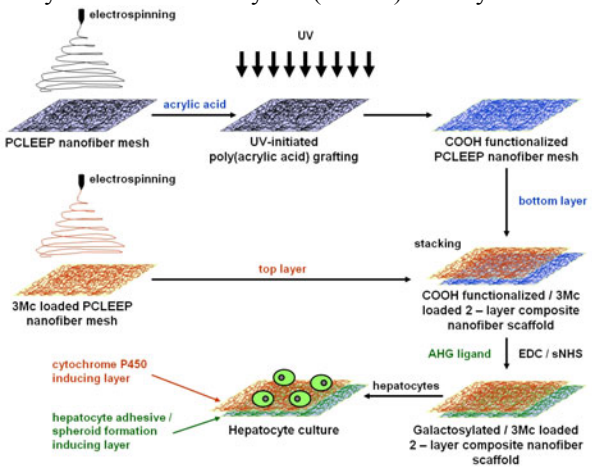

Figure 1: Nanofiber scaffold fabrication process.

Methods:

Poly(ε-caprolactone-co-ethyl ethylene phosphate) copolymer (PCLEEP, Mw: 112k) was electrospun into a nanofiber mesh. PCLEEP nanofibers were surface functionalized with carboxylic groups through UV-initiated poly(acrylic acid) (PAA) grafting. Another nanofiber mesh that was loaded with 0, 0.1, 1.0, 5.0 or 8.0 wt% 3Mc during electrospinning was stacked over the COOH-functionalized layer. An aminated galactose ligand, 1-O-(6'-aminohexyl)-D-galactopyranoside, was then conjugated onto the COOH-functionalized layer using carbodiimide crosslinking chemistry as described previously [1]. Subsequently, freshly isolated rat hepatocytes were seeded at 2×10^5 cells/cm^2 onto the various 3Mc loaded, galactosylated scaffolds and cultured for 7 days. For controls, hepatocytes were also seeded onto single layer galactosylated, non-3Mc loaded scaffolds and on tissue culture polystyrene (TCPS) and cultured in medium supplemented with and without 3Mc. Cultures were assayed for their cell attachment function, albumin secretion function (not shown) and P450-dependent ECOD activity [1].

Results and Discussion:

Hepatocytes cultured on various galactosylated, 3Mc loaded 2-layer composite nanofiber scaffolds exhibited similarly high hepatocyte attachment efficiency (74% - 81%, average 77%) 3 hours after cell seeding. The 3-Mc concentration in nanofiber scaffold did not influence cell attachment efficiency. Without, galactosylation (TCPS control), the attachment efficiency was poor (30%). In contrast, attachment was highest for single layer galactosylated scaffolds (84%), suggesting that for the double layered scaffolds, the upper non-galactosylated layer could slightly hinder cell attachment. Obviously, this conclusion will depend on the porosity and pore size of the top mesh. The effect was small in this case as the layer is highly porous and the cells could still interact with the lower galactosylated nanofiber layer.

On the double-layered nanofiber scaffold, the hepatocyte ECOD activity is strongly correlated with the 3Mc concentration loaded into the scaffolds (Figure 2). The P450-dependent ECOD function increased with 3Mc concentration, but decreased with time. This can be attributed to the gradual loss of 3Mc from the scaffolds. In contrast, P450 function of hepatocytes cultured on single layer galactosylated scaffolds with daily supplementation of 3Mc in the medium peaked at day 4.

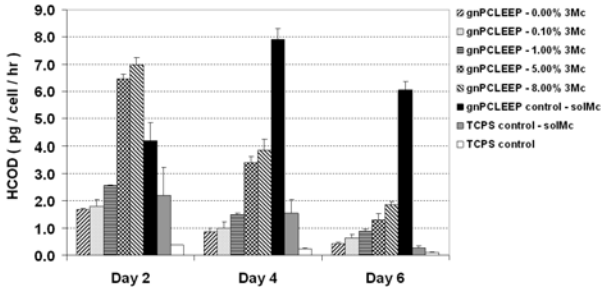

Figure 2: Cytochrome P450 function of hepatocytes cultured on various scaffold conditions.

Conclusions:

By taking advantage of the porous and layer-forming properties of electrospun nanofibers, we have designed a dual functional scaffold that induces two different biological responses from hepatocytes. The P450 function of the hepatocytes increased with the amount of 3Mc loading in the scaffolds. The mode of release of 3Mc to primary rat hepatocytes cultured on this dual function scaffold remains to be elucidated.

References:

[1] Chua KN. Biomaterials. 2005;26(15):2537-2547.

Acknowledgements:

This work is supported by A*STAR, NUS and Division of Johns Hopkins in Singapore.