Liver extracellular matrix-based nanofiber scaffolds for the culture of primary hepatocytes and drug screening

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Summary of work done

Acellularity of the liver tissues, post decellularization was confirmed by Hematoxylin and Eosin staining (H&E Staining), DAPI, and reduction of the DNA content. The presence of intact liver matrix proteins post-decellularization was confirmed by Masson Trichrome staining, Orcein, and Alcian Blue staining. The decellularized liver extracellular matrix (LEM) powder was mixed with the synthetic polymer solution, Polylactic acid (PLA) at different ratios, to fabricate electrospun hybrid nanofiber scaffolds (PLA-LEM). The SEM analysis of the fabricated hybrid scaffolds demonstrated uniform, homogeneous, bead-free smooth texture with cylindrical morphology constituting a dense network of fiber distribution with random orientation throughout the matrix. FTIR analysis confirmed the presence of proteins in the LEM-substituted PLA samples with additional peaks corresponding to the C-O stretching and N-H bending region denoting the presence of collagen and proteoglycans, which was not present in the PLA-only samples. The electrospun scaffold with only PLA depicted a contact angle of 106.7° whereas the PLA-LEM group showed a contact angle of 61.55°. Thus, it is evident that blending dLEM with PLA polymer significantly decreased ($P<0.0001$) the hydrophobicity of the overall electrospun matrix making it a perfect fit for the cells to adhere. The isolated primary hepatocytes 105 cells/cm2 of the surface were cultured on the fabricated PLA-LEM scaffolds for a period of 10 days and compared with PLA and RTC-coated plates as control conditions. Actin filament staining with Phalladoin showed the trans-differentiation of the hepatocytes cultured on the RTC-coated plates into fibroblast-like morphology after 3 days in cultures, whereas the hepatocytes cultured on the PLA-LEM scaffolds showed distinct hepatocyte characteristics with hexagonal morphology and multinucleated structures. The viability of the hepatocytes cultured on the fabricated nanofiber scaffolds was estimated with Calcein and Sytox viability staining. It was observed that the number of viable cells on the matrix containing hybrid scaffolds was higher than those cultured on RTC plates at all three-time points studied (Days 1,4 and 10). On day1, the number of live cells (Green) and dead cells (red) on both the RTC as well as PLA-LEM scaffolds were almost similar, the viability slightly reduced in the PLA group (79.15±1.41%) when compared to the

RTC group (90.2±1.24%) and PLA-LEM group (93.8±0.90%) at day4 and at day 10 we observed maximum viability of the hepatocytes cultured on the PLA-LEM group (83.8±3.0%) and was significantly higher than that of the RTC group (47.5±3.0%, $P<0.001$). A significant increase in CYP1A2 enzyme activity was observed in hepatocytes cultured on PLA-LEM hybrid scaffolds in comparison to those on collagen upon induction with phenobarbital. Drugs like acetaminophen and rifampicin showed highest toxicity with hepatocytes cultured on hybrid scaffolds. Also, lethal dose of these drugs in rodents was accurately predicted as 1.6 g/kg and 594 mg/kg respectively from the corresponding IC50 values obtained from drug-treated hepatocytes on hybrid scaffolds. Thus, the fabricated liver-specific electrospun scaffolds maintained primary hepatocyte viability and functionality for extended period in culture and serves as an effective ex vivo drug screening platform to predict an accurate in vivo drug-induced hepatotoxicity.