Electrospun Polycaprolactone Nanofibrous Scaffolds for Hepatocyte Culture

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Statement of Purpose: Tissue engineered structures are fast becoming the paradigm of choice for the repair and replacement of diseased and injured liver tissue. One of the attractive resorbable polymers for the preparation of porous scaffolds is polycaprolactone because it can be electrospun into nanofibrous webs. The objective of this study was to determine the optimal electrospinning conditions for preparing scaffolds that would support the culture and cellular growth of hepatocytes[1,2].

Methods: Polycaprolactone (PCL) (Mw 65,000) (Sigma-Aldrich) with a melting point of 60°C and a density of 1.145 g/cm^3, was dissolved in a 3:1 ratio of chloroform and methanol solvent. A spinning solution made by dissolving 10% by weight of PCL was used for all the electrospinning trials, which employed the use of a vertical parallel plate electrospinning system. The top plate was grounded, and the bottom plate was attached to the positive power supply of 48 kV, hence serving as the collection plate for the spun nanofiber web. A meltblown polypropylene nonwoven fabric served as the collection medium. Nanofibers were collected for a duration of 30 seconds each. Each sample was cut into circles, 35mm in diameter, and placed into 35mm culture dishes. Due to the hydrophobic nature of the polypropylene substrate, each sample was attached to the bottom of the culture dish with silicone type A medical adhesive glue (Dow Corning). After sterilization in UV light, each sample was soaked in culture media for 2 hours before cell seeding. The culture media used is Dulbeccos Modified Eagle Medium from Gibbs. The following are added to the medium: 10% fetal bovine serum, 20mM Hepes, 10mM nicotinamide, 30mg/L L-proline, 1mM L-asorbic acid 2-phosphate, 2μM dexamethasone, 1x antimycotic-antifungal, 0.5mg/L insulin-transferrin-selenium. The standard hepatocyte American Type Culture Collection cell line AML12 (ATCC Catalog No. CRL-2254) was used following a static culture technique[3]. Samples were incubated at 37°C and 5% CO_2. The presence and density of cells attached to the nanofibrous scaffolds were observed using scanning electron microscopy. For a live/dead cell viability test the fluorescence cytotoxicity assay for mammalian cells was used. Samples were tested after one week in culture and after the stain had been incubated for 25 minutes. The polypropylene melt-blown fabric with no nanofibrous web served as the control. The results are reported in Table 1.

Results/Discussion: After 7 days in culture, the initial tests showed a strong growth of hepatocytes (Figure 1). The live/dead assay stained the cytoplasm of live cells green, while dead cells were identified as red. The dead cells were shown to be smaller in size as only the nucleus was stained red. In terms of the number of live hepatocytes present, the fluorescence assay exhibited similar density and morphology for both the scaffolds and control sample (Table 1). SEM images confirm that the cells are attached to the PCL nanofibrous scaffolds.

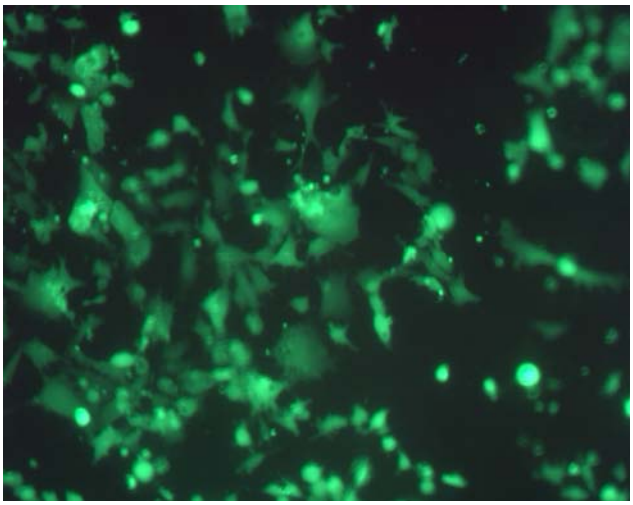

Figure 1: Live hepatocytes on PCL nanofibrous scaffolds after seven days in culture (Mag x10).

SEM images confirm that the cells are attached to the PCL nanofibrous scaffolds. The average diameter of the nanofibers was 225.7 nanometers, with a standard deviation of 29.1 nanometers.

Substrate Used	**Average Number of Live Cells**
Control	252.54, stdev 0.98
PCL nanofibrous scaffold	253.41, stdev 1.12

Table 1: Average number of live cells present after seven days of culture

Conclusions: The initial results showed positive cell growth on the PCL nanofibrous scaffolds. The hepatocytes were found to proliferate similarly to those cultured on the control surfaces without nanofibers. The scaffolds will be used to encourage hepatocyte grow on a substrate that can be used in tissue engineering and organ regeneration. The next step in the research will be to measure the nanofiber properties such as, fiber diameter, overall porosity, and pore size distribution. Then, we will be able to determine how the nanofiber web properties influence hepatocyte proliferation. Research will also focus on whether the cells can penetrate the scaffolds and grow into the webs. Further studies will be undertaken to determine the viability of liver cells. Concurrently, the use of a dynamic culture technique will also be investigated.

References:

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