Alginate Based Electrospun Nanofibrous Hepatocarcinoma (HepG2) 2D Cell Culture Model

Lina Wu, Driton Vllasaliu, Bahijja Tolulope Raimi-Abraham

School of Cancer and Pharmaceutical Sciences, King's College London, London SE1 9NH, UK

Background: Functionalized electrospun nanofibrous has attracted interest in various drug delivery, tissue engineering, and cell culture applications. In tissue engineering, electrospun nanofibres are often used as synthetic scaffolds to mimic the extracellular matrix (ECM). Greater biomimicry can be achieved using natural polymers such as collagen and elastin fibres which are naturally present in human ECM providing a good correlation to the in vivo environment. The work presented here aimed to determine the effect of alginate addition on spinnability and HepG2 cell viability. A combination of natural and synthetic polymers was used namely polycaprolactone (PCL), alginate (ALG), and poloxamer 188 (P188) and their effects on HepG2 cell viability were evaluated. PCL is well known to possess desirable properties suitable for mechanical characteristics, such as a slow degradation rate and higher elastic modulus. P188 is a non-ionic surfactant that can improve cell adhesion and proliferation. ALG is a natural polymer that is widely used in various pharmaceutical and medical applications due to its outstanding biocompatibility, solubility, porosity, and degradability. The human hepatocellular carcinoma cell line HepG2 has many liver-specific functions and has wide use for drug screening, hepatoxicity and study of the metabolism of xenobiotics.

Methods: The following polymer solutions PCL, PCL/P188/ALG (89:10:1), and PCL/P188/ALG (88:10:2) with a total polymer concentration of 10% in acetone were prepared. Nanofibres were generated by electrospinning at voltages of 15 kV, 18kV, 18kV respectively and at a 2 mL/min flow rate. Residual solvent within the resultant nanofibres was evaluated by moisture analyzer. Nanofibre diameter (Image J) and morphology were evaluated using scanning electron microscopy (SEM), and physicochemical characterisation using attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy. HepG2 cell viability was determined using MTT assay on day 2 and 4. Cell viability is presented as % absorbance compared to control (cells cultured on tissue culture multiwell plate plastic).

Results: PCL, PCL/P188/ALG (89:10:1), and PCL/P188/ALG (88:10:2) nanofibres were successfully generated by electrospinning. The residual solvent content were 0.5%, 2.2% and 11.1% respectively. SEM images showed that all the nanofibres have smooth surfaces and cylindrical shapes. The diameters were 274±43nm, 820±250nm, and 814±171nm respectively. The presence of ALG increased the average diameter of the nanofibers. ATR-FTIR studies on both PCL/P188/ALG nanofibres showed that the peaks corresponding to P188 (~2881 cm^{-1}, and ~1100 cm^{-1}), along with the peaks corresponding to the PCL(~1700cm^{-1}) and the peaks corresponding to alginate (~1600cm^{-1}) confirmed the successful blending of each component. MTT assay showed that the effect of ALG on cell viability was not obvious on Day 2. However, the PCL/ P188/ALG (88:10:2) scaffold exhibited 29% higher cell proliferation on day 4 when compared to the scaffold with PCL/P188/ALG (89:10:1) and 35% higher than the control group.

Conclusions: This study succesfully generated electropun alginate based nanofibres more specifically PCL/P188/ALG (89:10:1) and PCL/P188/ALG (88:10:2). All nanofibres generated showed improved cell viability compared to control. The addition of ALG and increased concentration resulted in greater cell viability on day 4. Further optimization of the ALG concentration will be conducted to obtain the best proliferation effect of the scaffolds.