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Multimaterial tandem electrospinning for spatially modulated neural guidance

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The goal of this work is the creation of an in vitro platform to investigate the combined effects of patterned topographical and bioactive cues towards achieving the spatially controlled growth of peripheral sensory neurons. The platform is intended to mimic the nanofibre topographical guidance characteristics of the native extracellular matrix and employ material and biofabrication strategies able to translate results to an implantable scaffold. To achieve a suitable platform the new Tandem Electrospinning technique was developed in order to create spatially defined multimaterial patterns of different populations of oriented nanofibres. Scaffolds were created using two polymer solutions of a PEOT/PBT copolymer dissolved in chloroform/HFIP with both solutions simultaneously electrospun onto a patterned target electrode. The formation of nanofibres was confirmed through scanning electron microscopy and hydrophobic fluorescent dyes were added to each solution to visualize the distribution of each population. To create spatially modulated growth orthogonal copper-click chemistry and maleimide/thiol conjugation additives were added to polymer solutions prior to electrospinning. Confirmation of selective conjugation was performed using ToF-SIMS and functionalized fluorescent dyes. Two laminin peptide sequences GRGDS and p20 were selectively conjugated to the two different fibre populations respectively and ToF-SIMS was used to verify their presence. Dorsal root ganglians (DRGs) from 1 day-old Wistar rat pups were explanted and placed at the overlapping region of the two fibre populations followed by microscopy analysis of fluorescence immunohistochemistry staining for ß(III)-tubulin after 5 days. Through the tuning of electrospinning parameters and target collector design it was possible to achieve a single step deposition of multiple fibre types within a confined space. Each population of fibres exhibited an aligned orientation and there was an observed region of overlap between the fibre types. The presence of selective functional groups on the fibre surface from the conjugation additives was confirmed through the application of functionalized dyes. Successful conjugation of peptide sequences to the fibre surface was also confirmed via ToF-SIMS. Initial in vitro results of neurite outgrowth from explanted DRGs suggest that the oriented nanofibre topography is able to promote directed neurite extension while the regionally defined biofunctionalization is capable of spatially modulating neurite growth. These results validate the newly developed Tandem Electrospinning method to create an in vitro platform that exhibits nanofibre topographical guidance cues and selective functionalization able direct and spatially modulate the growth of neural cells. This work was partly funded by NSERC of Canada.