Generation of tissue scaffolds composed of aligned electrospun fibres of interpenetrating polymer networks of silk fibroin and PEDOT:PSS for peripheral nerve regeneration

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Introduction: Over 1 million people worldwide suffer from trauma and peripheral nerve injury (PNI). Electrical stimulation (ES) is a method for peripheral nerve regeneration and nerve conduits are another [2]. ES can be used in therapeutic purposes for the relief of nerve pain, and it can also activate neurite outgrowth of neuronal cells *in vitro* [3,4]. Therefore, the integration of ES with an electrically conductive nerve conduit may accomplish the regeneration of fully functional nerves post trauma effectively. Here, we describe the development of electroactive composites of silk fibroin (SF) and poly(3,4-ethylenedioxythiophene)-polystyrene sulfonate (PEDOT:PSS) as interpenetrating polymer networks (IPNs) of sub-micron fibres as novel peripheral nerve tissue scaffolds.

Methods: Materials composed of non-woven mats of sub-micron fibres were fabricated based on double layers of electrospinning SF in formic acid (FA) and calcium chloride ($CaCl_2$). The base layer of electrospun material was composed of randomly aligned fibres, whereas the top layer was aligned fibres, with thicknesses of 100 and 10 µm, respectively. The electrospun SF materials were treated with 80% ethanol (EtOH) to induce β-sheet formation. Next, the EtOH-treated fibres were soaked in a solution of EDOT monomer, PSS and an initiator for 3 days. The concentration ratio between EDOT and PSS (α) was varied from 1.3 to 3.3, to form interpenetrating polymer networks (IPNs). The physical and mechanical properties of these materials were characterized by scanning electron microscopy (SEM), hard X-ray photoelectron microscopy (HAXPES), and tensile testing. Finally, the optimal conditions of PEDOT:PSS modified fibres were coated with laminin, their cytotoxicity and biocompatibility with the neuron-like cell line (NG108-15) were tested. Cell viability, metabolic activity, DNA concentration, and neurite extension length were ascertained for 7 days.

Results: The fibre diameter of electrospun materials was 190 ± 50 nm in both layers and no significant difference was observed after treatment with EtOH or chemical modification with PEDOT:PSS. The PEDOT:PSS modified fibres were turned dark blue. Moreover, HAXPES results revealed that there was a significantly higher atomic percentage of Sulphur (S) in the IPNs compared to the unmodified SF fibres. The stain at break and toughness of IPN materials were significantly decreased when α = 2.8 and 3.3. Additionally, there was no toxicity from IPN materials in the conditions of α = 2.3, 2.8, and 3.3. Cell metabolic activity and DNA concentration of NG108-15 cultured on SF and IPN materials were steadily increased from day 1 to day 7.

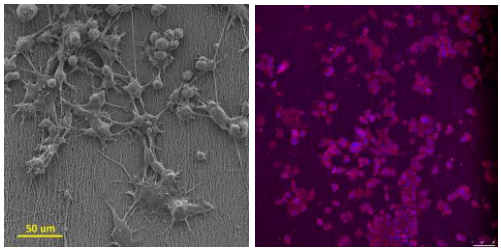

Figure 1. NG108-15 on PEDOT:PSS modified electrospun SF (α = 3.3) coated with laminin: (left) SEM and (right) membrane stain – red and nucleus stain – blue

Discussion & conclusion: The sub-micron aligned SF fibre has quite similar diameter to the neurite. These fibres are fragile in the dry state but flexible when hydrated due to plasticization of the SF by water. HAXPES and cytotoxicity results suggest that the electroactive SF:PEDOT:PSS IPNs are biocompatible. Moreover, the electroactive fibres can support neural cell proliferation and also neurite outgrowth when coated with laminin. The electrical conductivity of the fibres and its relation to external electrical stimulation regimes for enhanced neurite extension lengths will be studied in depth in the future.

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References

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