Using phosphate glass fibres to improve the interface between damaged peripheral nerve and engineered neural tissue

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INTRODUCTION: Advances in biomaterials and tissue engineering have led to the development of Engineered Neural Tissue (EngNT) peripheral nerve repair [1]. Experiments using EngNT with embedded Schwann cells to repair rat sciatic nerve injuries indicated sub-optimal growth of neurites from the proximal nerve stump into the EngNT [1, 2]. Phosphate glass fibres (PGfs) have been used in hard- and soft-tissue engineering applications [3, 4]. They are biocompatible and biodegradable and have emerged as a potential material to resolve soft-tissue engineering interface issues [5]. The aim of this study was to investigate whether PGfs could improve the interface between the proximal stump of a damaged nerve and EngNT in supporting neurite outgrowth.

METHODS: Bovine collagen gels (2 mg/ml) containing $4x10^6$ SCL4.1/F7 cells per ml were made in purpose-built PEEK moulds. To create gels that were incorporated with PGfs, 150 mg of PGfs (with a molecular composition of $50P_2O_5$-$40CaO$-$5Na_2O$-$5Fe_2O_3$) were placed into the mould prior to casting in the gel. The fully hydrated cellular gels with and without PGfs were stabilised using RAFT™ absorbers (TAP Biosystems) thereby creating EngNT and EngNT+PGfs. Dorsal root ganglia were extracted from genetically modified, green fluorescent protein positive Sprague Dawley rats (200-250 g), culled by CO_2 asphyxiation. Excised DRGs were cut into 2 halves. The freshly cut surface was placed in direct contact with the surface of EngNT either with or without PGfs. EngNT or EngNT+PGf with DRGs attached were placed vertically into 1.5ml tubes and maintained in a tissue culture incubator (37°C/5% CO_2) for 72 h with DMEM medium supplemented with penicillin streptomycin and foetal bovine serum. Measurements of axonal growth into the construct (detected by immunostaining for β-III tubulin) and satellite glial migration were performed using ImageJ.

RESULTS: *Table 1. Average maximum and average mean distance of satellite glia cell migration into construct. Data are means ± SEM, n=6, t-test revealed ***p<0.001 and **p<0.05 for average maximum distance into construct and average mean distance into construct, respectively.*

	EngNT	EngNT + PGfs
Maximum distance into construct (μm)	149 ± 33	874 ± 132
Mean distance into construct (μm)	281 ± 13	400 ± 26

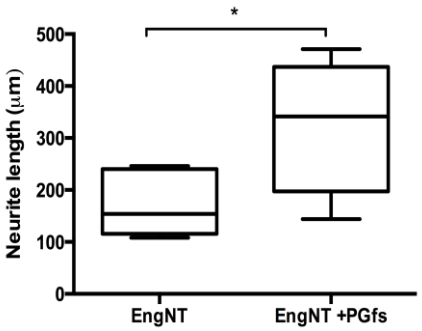

*Fig. 1: Box plot showing the lengths of neurites measured in EngNT and EngNT + PGf, where n=3, t-test revealed *p<0.01*

DISCUSSION & CONCLUSIONS: This study demonstrates that PGfs can be successfully incorporated into EngNT to encourage satellite glia migration and neurite elongation. *Table 1* shows that incorporating PGfs permits satellite glia cells to travel approximately one and half times further into the construct. *Figure 1* shows that neurites are able to elongate approximately twice the distance into EngNT that was modified with PGfs. Further work involves testing the inclusion of PGFs into EngNT constructs tested in vivo to see whether ingrowth of neurites from the proximal nerve stump is improved.

REFERENCES:

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