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ALIGNED CELLULAR AND ACELLULAR COLLAGEN GUIDANCE SUBSTRATES FOR PERIPHERAL NERVE REPAIR

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INTRODUCTION: There is a clinical demand to shorten the delay of reinnervation and improve functional recovery after peripheral nerve injury. A peripheral nerve repair device with the ability to direct and promote axon growth across a lesion would be a promising alternative to nerve autograft repair, the current gold standard treatment. The growth of axons across a lesion is most effective when supported by columns of aligned Schwann cells, as found in an autograft. Here we report the development of a robust aligned cellular collagen biomaterial that supports and directs neuronal growth. We also investigate the potential of these aligned cells to precondition the collagen biomaterial, before they are freeze-killed, leaving an acellular guidance matrix.

METHODS: The rat Schwann cell line SCL 4.1/F7 was used throughout. Cells were mixed with neutralised type I rat tail collagen (2 mg/ml). As gels formed they were tethered to permit cellular alignment¹. 24 hours later, gels were plastic compressed (PC) by the rapid removal of the interstitial fluid from fully hydrated gels. The preconditioned acellular matrix was prepared in the same way but the compressed gels were then submerged in liquid nitrogen to kill the cells². To investigate neuronal growth on these aligned cellular and preconditioned acellular biomaterials, adult rat dissociated dorsal root ganglia (DRG) cells were cultured on their surfaces for 3 days. Plastic compressed collagen matrix in which no cells had been seeded was used as a control. In all cases, neurite growth was quantified using immunostaining, with anti-S100 and anti- β III tubulin, and confocal microscopy.

RESULTS: Around 70% of DRG neurons growing on the aligned cellular biomaterial did not deviate from the angle of Schwann cell alignment by more than 20 degrees (Fig 1A). Less neuronal growth was observed on the preconditioned acellular matrix (Fig 2B), compared to the aligned cellular biomaterial (Fig 1A & B). Pilot data suggest growth of regenerating neurites on the acellular matrix was guided by the direction of the previous cellular alignment (Fig 2B) compared to control.

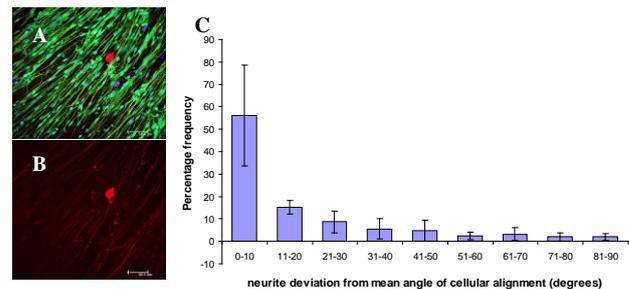


Fig. 1: Aligned Schwann cell biomaterial guides neurite growth. **A** Confocal micrograph projection showing neuronal growth on the surface of the aligned Schwann cell biomaterial. **B** Red channel only (neurite growth). **C** Deviation between angle of neurite growth and Schwann cell alignment (means \pm SEM)

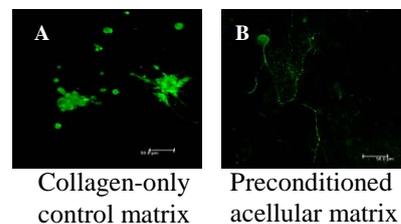


Fig. 2: Confocal micrograph projections showing neuronal growth on collagen-only control (A) & preconditioned acellular (B) matrices.

DISCUSSION & CONCLUSIONS: Aligned Schwann cells within a PC 3D collagen matrix promote and support neuronal growth in a directed manner. This occurs whether the Schwann cells are alive or dead, although neurite growth is more robust on a live Schwann cell substrate.

We are currently integrating the aligned cellular biomaterial into a repair device. The matrix is rolled into columns¹ which are then packed together within a silicone tube. Testing this 'engineered endoneurium', using *in vitro* and *in vivo* models, will demonstrate the potential of the device as an implantable conduit for peripheral nerve repair.

REFERENCES: ¹ E. East et al (2010) *Tiss Eng Part A* **16**: 3173-3183. ² E. Hadjipanayi et al. (2010) *British Journal of Surgery* (pp. S37-S37).

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