Plastic Compression of Aligned Cellular Collagen Gels for Nervous System Repair

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Introduction. Implantation of tissue engineered bridging devices provides a promising therapeutic area for treatment of nervous system injuries. Alignment of glial cells within collagen gels enhances axonal growth and provides a directional cue for regenerating neurons in both central and peripheral nervous system repair [1, 2]. Collagen gels do not lend themselves to direct implantation due to poor mechanical handling properties, but subjecting them to plastic compression produces dense, mechanically robust structures [3]. By combining alignment with compression, a potentially useful device can be produced. The aim of this study was to investigate glial cell survival following plastic compression, and to investigate whether cellular alignment was retained.

Materials and Methods. To assess the effect of plastic compression on glial cell viability, cells were seeded into collagen gels and compressed for 1 or 5 min. Cell death was assessed using propidium iodide and Hoechst immediately or after a further 20 h in culture and compared with control gels. In alignment experiments, glial cells expressing green fluorescent protein (GFP) were seeded into collagen gels tethered at both ends [2]. Following contraction and cellular alignment, gels were compressed then rolled (Figure 1A). Dissociated DRG neurons were cultured on these constructs for 3 days, before immunostaining for βIII tubulin. Images were captured using an Olympus BX61 microscope, or a Leica DMIRBE confocal microscope.

Results. Plastic compression of collagen gels increased glial cell death only slightly compared to uncompressed controls, regardless of time in culture post-compression (Figure 1B). Self-aligning GFP glial gels were released from their tethering bars following contraction of the gels (48 hrs), and were compressed for 1 min. Following plastic compression, the glial cells retained their alignment (Figure 1 C and D), and provided guiding processes along which neurons could grow (Figure 1 E and F).

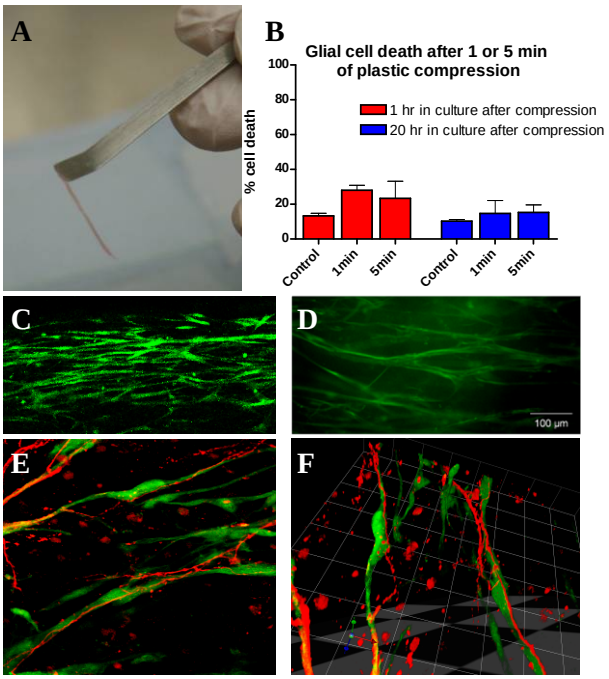

Figure 1. ***A****. rolled up aligned glial device following plastic compression. The majority of glial cells survived plastic compression (**B**), glial alignment was retained following compression (**C** & **D**), and supported guided neuronal growth (**E** & **F**).*

Discussion and conclusions. Plastic compressed aligned glial devices support neuronal growth in a directed manner and have improved mechanical handling properties compared to uncompressed collagen gels. Rolling flat sheets of aligned glia into thin rods provides a means to engineer fibres of aligned cells which could be packed together to form an implantable conduit for nervous system repair.

References [1] East E. et al (2009) *Glia* **57** (S13) S159. [2] Phillips J.B. et al (2005) *Tiss Eng* **11** 1611-7. [3] Brown R.A. et al (2005) *Adv Funct Mater* **15** 1762-70.

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