Effect of collagen gel concentration gradients on neurite elongation

C.Kayal[1], R. Shipley[1], JB. Phillips[2]

[1]*Department of Mechanical Engineering, University College London* [2]*Biomaterials and Tissue Engineering, University College London*

INTRODUCTION: Collagen represents the main component of the extracellular matrix for peripheral nerves and is commonly used in tissue engineering to make conduits for nerve repair. However, little is known about the effect of the mechanical properties of collagen on the extension of neurites[1]. This study focuses on quantifying neurite extension in response to defined collagen gel gradients. Subsequently, experimental data will be integrated in a mathematical model to inform the design of tissue-engineered conduits combining both mechanical and chemical gradients in order to improve peripheral nerve repair approaches.

METHODS: PC12 cells were cultured *in vitro*, in the presence of nerve growth factor (NGF)[2] on the surface of collagen gel gradients. Stabilised collagen gel gradients were fabricated using rat tail collagen type I (First Link). A standard protocol[3] was used to generate neutralised collagen solutions (2 mg/mL) that were incubated at 37º for 10 min in 24-well plates at an angle[4] of 45 ˚ from the horizontal plane. The resulting wedge shaped gels were stabilised using RAFT™ absorbers (TAP Biosystems) to produce gradient gels. PC12 cells (5 x 10^4 cells/well) were seeded onto the upper gel surfaces, left to adhere for 1h at 37º, then culture medium added (supplemented with 0.663µg/mL NGF)[2]. Cultures were maintained at 37º, 5% CO_2 in a humidified incubator for 3 days. Different media formulations were compared, including conditioned media from C6 glioma cells and RPMI media supplemented with 10% serum.

To quantify the impact of the physical gradient on neurite growth, we used a collagen gradient with a predefined (left to right) density gradient from 55 mg/mL to 70 mg/mL. Each gel was separated into two parts, the lower density range section and the high density range section, for analysis. Gels were fixed and neurites were visualised using immunofluorescence labelling and confocal microscopy. Length and orientation of neurites were measured using ImageJ. For the orientation, the angle of each neurite in relation to the orientation of the gradient was determined (Fig 1).

RESULTS: On the lower density range gradient gel sections, 74.35% of neurites grew from the low density to the high-density regions of the gels. By comparison, neurites on the higher density range gradient gel sections showed no preferential direction (50% grew towards the lower density gel regions) (Fig 1). Further, neurites were longer on the high collagen density gel sections, with a cumulative neurite length of 2830 µm, which was reduced to 1720µm for the low collagen density gel sections.

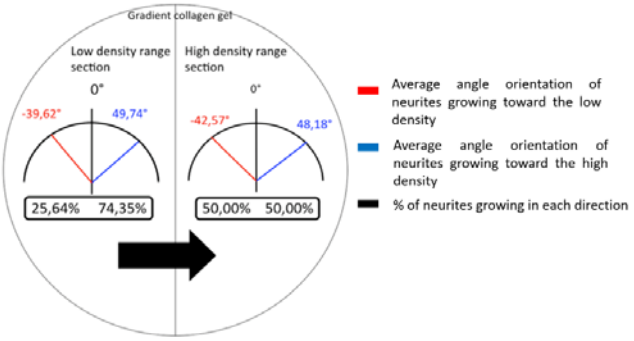

Fig.1: Mean orientation of the neurites depending on their position on the collagen gel gradient (high or low part of the gel), taking into account results for both media formulation conditions

DISCUSSION & CONCLUSIONS: This study shows that neurite orientation and length can potentially be influenced by physical gradients within collagen gels *in vitro*. In future, we will quantify the stiffness gradients within the gels[1] to correlate the stiffness gradient to the neurite extension. Mathematical modelling using these data will then be used to simulate how neurite extension might be improved and controlled[2]. These results, if extended to the *in vivo* situation, would assist significantly in the design and fabrication of constructs for neural tissue engineering in the future.

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