Optimizing Schwann Cell Migration Using Laminin Derived-peptides to Improve Nerve Regeneration

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Statement of Purpose: Peripheral nerve injury is a condition in which nerves are damaged as a consequence of physical distress from a traumatic injury. Despite advances in the reconstruction of segmented nerves, treatments are still inadequate in providing functional and efficient repair, with axon regeneration particularly limited in critical sized defects (*i.e.*, greater than 3 cm in humans). A main priority, therefore, is investigating new clinical solutions including acellular allografts that optimize the healing process by enhancing the innate response. Nerve repair is mediated largely by Schwann cells (SC), the principal glia that supports neurons in the peripheral nervous system. Axonal regeneration *in vivo* is limited by the extent of SC migration into the nerve conduit, where SC assist in the clean up processes, release growth factors, and myelinate the regenerated nerve. Thus, inducing directional migration of SC across the synthetic nerve guide using haptotactic cues would be highly advantageous. Herein, we have characterized the influence of laminin derived-peptides – such as RGD, YIGSR, RNIAEIIKDI, IKVAV and PDSGR - on Schwann cells behavior, identifying their potential to promote cell guidance under a peptide concentration gradient.

Methods: Vinyl-terminated one-dimensional concentration gradient substrates were fabricated by vapor deposition using 5-hexenyldimethylchlorosilane and 25 mm^2 glass slides, in a confined channel diffusion method. Peptide concentration gradients were achieved by thiol-ene "click" reaction, using cysteine terminated peptides. The vinyl end group gradient profile was measured by static water contact angle, and survey spectra (0-700 eV) from XPS confirmed the thiol-ene reaction, with data collected from five equidistant points on the surface at 5 mm intervals. Decomposition of peak fitting of XPS C_{1s} high resolution spectra for each gradient position based on Gauss-Lorentz function was analyzed to achieve surface concentration and coverage fraction as a function of position along the gradient surface. Schwann cell lines isolated from adult rat sciatic nerves were used for this study. Purification and culture were based on a selective culture media, supplemented with forskolin, N_2 and D-valine. Cells were seeded on the samples and controls (uniform self-assembled monolayer of respective peptides) at a concentration of $1x10^4$ cells/cm^2 for 4 h in a serum-free media to eliminate adsorbed protein interfering with the initial cell attachment, and then cultured in 10% FBS media for 1 and 3 days. Samples were fixed and labelled with rhodamine phalloidin for actin, vinculin for focal adhesion, and DAPI for nuclei (n=3). A MATLAB code based on edge detection was utilized to determine actin alignment.

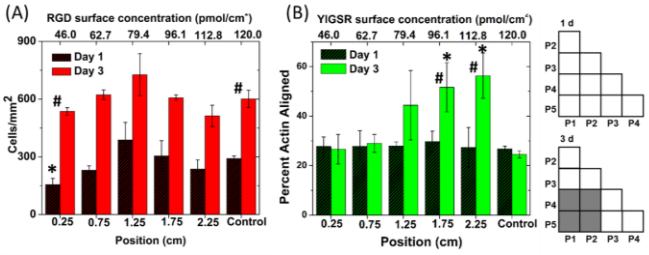

Figure 1. Influence of concentration gradient of laminin derived-peptides on (A) proliferation with RGD and (B) alignment with YIGSR, respectively. Values are represented as mean ± S.E., with $P < 0.05$. ANOVA with Tukey's, $P < 0.05$. Grey shaded boxes indicate significant differences from different positions at the same time point, "#" different time points at the same position and "*" statistically significant differences with respect to control.

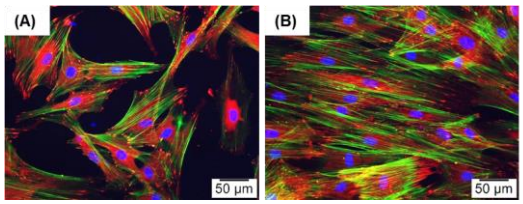

Figure 2. Immunofluorescent staining of vinculin (red), actin (green) and nuclei (blue) at (A) day 1 and (B) day 3 of Schwann cells culture in a YIGSR gradient sample.

Results: Laminin, the major extracellular matrix protein for nerve regeneration, has several bioactive epitopes that have been identified to promote cell attachment and/or neurite outgrowth and axon guidance. Such peptides have the advantages of higher stability compared to the native protein laminin. We created concentration gradient of several laminin derived-peptides and studied their influence on SC behavior. As expected, different peptides promoted different responses from the cells. For example, as RGD promoted cell adhesion and proliferation (Figure 1A), YIGSR promoted cell alignment to the direction of the gradient of concentration (Figures 1A and 2), especially at higher concentrations of the gradient.

Conclusions: The results show the potential of using laminin derived-peptide gradients as an additional and valuable cue on creating scaffolds for nerve regeneration. By selecting an appropriate combination of peptides, it is possible to tune the Schwann cell response, promoting cell adhesion, proliferation and migration according to the concentration gradient direction. Future work will focus on using functionalized nanofibers with a gradient concentration of combined peptides as a synthetic nerve guide to induce directional migration of SC by means of topographic and haptotactic cues.

References:

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