

Novel Neural Interface for Vision Prosthesis Electrodes: Neurite Outgrowth through Biomolecule Incorporation

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INTRODUCTION: Epiretinal vision prostheses aim to restore vision to patients blinded by photoreceptor loss as a result of prevalent diseases retinitis pigmentosa (RP) and age related macular degeneration (AMD). Close apposition of retinal cells with implanted electrodes is critical to restoring vision¹. Conductive polymers, such as polyethylene dioxythiophene (PEDOT) coated on metal electrodes are proposed to improve the electrode interface by decreasing strain-mismatch and bi-layer capacitance that occurs between metal electrode surfaces and neural tissue. This study proposes incorporating peptide dopants in conductive polymers to provide sites conducive to cell attachment. Several laminin peptides have been identified to target cell adherence (such as YIGSR and RGD) and neural growth or repair (YFQRYLI and SIKVAV)². This research explores two of these laminin peptides (YIGSR and YFQRYLI) combined with incorporation of nerve growth factor (NGF) into films. The aims were to assess the differentiation of cells on NGF incorporated films compared to differentiation of cells in NGF supplemented media and additionally to assess neurite cell adhesion on peptide doped films compared with laminin coated control films.

METHODS: Solutions of 0.1M EDOT doped with 0.05M pTS, 5µg/mL DCDPGYIGSR or 5µg/mL DEDEDYFQRYLI and supplemented with 1µg/mL NGF were produced in a solution of DI water and acetonitrile. The films were formed on platinum electrodes by galvanostatic electrodeposition at 2.0 mA/cm² for 5 mins.

Films were washed with DI water for 12 h at 37 °C, disinfected by immersion in 70% EtOH and placed under UV for 1 hr. 5 µg/mL laminin from murine sarcoma was coated on PEDOT/pTS controls. Fluorescent PC12s were plated at 20000 cells/cm², in 1% horse serum, RPMI. NGF was used as a media supplement at 50ng/mL in control films. Fluorescent images were taken at 96hrs and results were analysed using NeuronJ software. Average neurite length was used to assess cell differentiation and cell density was used as an indication of cell adherence.

RESULTS: Neurite outgrowth stimulated by NGF within the polymer film provided sufficient stimulus to result in neurites of comparable average length to those grown using NGF

supplemented media (see Fig 1). The average neurite length across all substrates was between 57µm and 75µm, with no significant difference between the application types of NGF.

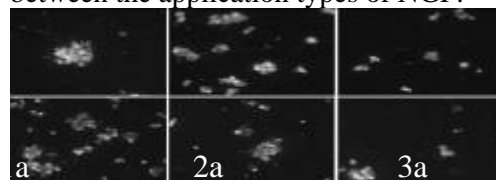


Fig 1: PC12s at 96hrs: 1.PEDOT/pTS; 2.PEDOT/DCDPGYIGSR;3.PEDOT/DEDEDYFQRYLI. a. NGF supplemented media and b. NGF incorporated into films.

Cell densities were much lower for films incorporating peptides indicating that exposure of adhesion peptides in doped films was less effective than in films coated with whole laminin (see Fig 2). However, films that incorporated peptides as the dopant grew neurites that were of similar length to those on laminin coated controls.

Fig 2: Cell density at 96 hrs.

DISCUSSION & CONCLUSIONS: This study shows that NGF contained within a polymer film can stimulate neurite outgrowth in PC12s. The use of laminin peptides as a dopant provided inferior adhesion of the PC12s than when the whole laminin molecule was used as a surface coating. This could be due to the laminin peptides being at a concentration that is too low, or accessibility of the peptides to cell receptors being limited by their restriction in the polymer matrix. Future studies will examine optimising presentation of peptide dopants.

REFERENCES: ¹Lovell, N.H., et al., *Neural Engineering/Neuro-Nanotechnology*, 2006. ²Nomizu, M., et al., *J Bio. Chem*; Vol. 273:46; 2006.

