Preclinical evaluation of neural interfacing electrode materials for bionic devices

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Statement of Purpose: Conventional metals such as platinum (Pt) have limited cell interaction due to their mechanical stiffness and smooth surface. The poor integration *in vivo* leads to fibrous encapsulation and limits Pt efficacy and safety of implantable electrodes [1]. Recently, conductive polymers (CPs) and conductive hydrogels (CHs) have been investigated for use as coatings for stimulating tissue in implantable bionic devices. While CPs can greatly improve the electrical properties of bioelectrodes [2], the expected improvement to cell integration has not been well supported *in vivo* [1]. CHs have been shown to reduce interfacial stiffness [2], but little is known about the cell interactions and *in vivo* performance. This study aimed to understand *in vitro* and *in vivo* cell and tissue interactions of new CH in comparison to conventional CP and Pt.

It is critical to the understanding of cell and tissue response to use a range of approaches to evaluate the specific interactions of relevant, functional cell types as well as the potential for inflammation. This is due to the need for the material to perform safely with minimal adverse host response and the requirement for stimulating devices to appropriately activate target neural tissues. Neural cell types including the clonal pheochromocytoma cells (PC12s) [3], primary dorsal root ganglia [4], Schwann cells [5] and primary spinal neurons [6] have been used to perform *in vitro* evaluation of bioelectrode materials. However, each cell type has different physicochemical cues which support optimal interactions. Additionally, *in vivo* standards for preclinical testing require intramuscular (IM) or subcutaneous implantation. This study assessed electrode material interactions using both PC12s and primary spinal neurons, in single cell and in co-cultures with supporting glia. An *in vivo* IM study was used to examine chronic encapsulation of materials.

Methods: CPs and CHs were fabricated using methods previously reported in our labs [2]. The electrodeposited CP poly(3,4ethylene dioxythiophene) (PEDOT) doped with pTS and the CH, PEDOT grown through a biosynthetic hydrogel (16 wt% polyvinyl alcohol crosslinked with 2 wt% heparin and 2 wt % gelatin) were prepared as detailed in [2] and compared with Pt.

PC12 and Schwann cell studies were conducted over 5 days under neuronal differentiation conditions with nerve growth factor in low serum media. Immunofluorescent microscopy was used to measure cell and neurite density. Primary myelinating spinal cord neurons were prepared from fetal murine spinal cord using published methods [7]. Cultures were grown for 3 weeks before fixing and immunostaining for axons and myelin. Primary astrocytes were derived from murine neonatal forebrain.

CH and CP coated Pt pins were implanted in the rabbit paravertebral muscles. Samples were retrieved at 12 weeks, resin embedded and stained with toluidine blue.

Results: The electrode materials used in this study each have a distinct surface topography, as shown in Fig 1.

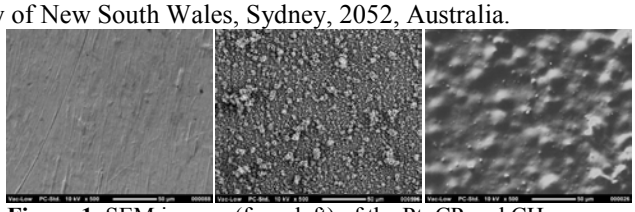

Figure 1. SEM images (from left) of the Pt, CP and CH

The clonal PC12s were compatible with both CPs and CHs. The CHs supported more cell attachment than CPs ($28x10^3$ compared to $17x10^3$ cells/cm^2), but both had significantly higher cell numbers than the Pt ($9x10^3$ cells/cm^2). The CH contains gelatin which can improve cell attachment through active peptide binding. The rough topography of the CP is also thought to encourage cell attachment. When cultured with Schwann cells all materials had increased PC12 attachment and neurite outgrowth compared to homogenous cultures (Fig 2), but no discernible difference was seen between CPs and CHs.

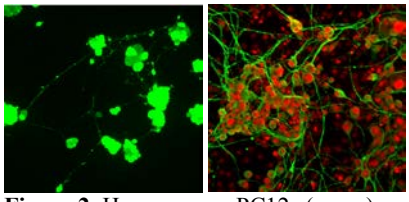

Figure 2. Homogenous PC12s (green) vs co-culture with Schwann cells (red)

Homogenous spinal neurons had poor or no attachment on any substrate. Neuron attachment and survival was improved when astrocytes were grown on the polymers prior to plating the spinal cord cells. The best axonal growth from co-cultured neurons was found on the CHs, but growth was generally considered poor on all electrode materials.

Minimal encapsulation was observed *in vivo* at 12 weeks (Fig 3) with no significant difference between the CPs and CHs. While encapsulation was not increased around the Pt controls, there was a noteable gap between the implant and tissue. There was evidence of CP delamination, but the CH was well integrated and stable.

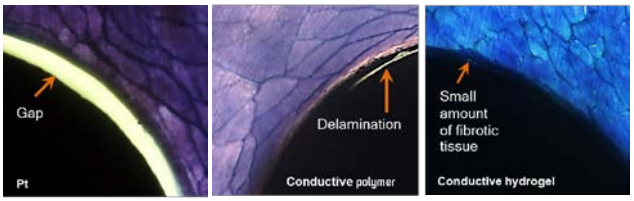

Figure 3. *In vivo* encapsulation of electrode materials (12 wks)

Conclusions: It is clear that different *in vitro* cell systems produce diverse biological responses to these materials. Co-cultures of either clonal or primary cells showed improved cell attachment and differentiation, but less variation between materials. This was reflected in the *in vivo* study. The CH was shown to be a promising option for improving bioelectrode properties. Future *in vivo* studies in contact with neural tissue are essential.

References:

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