An Improved Model for High-throughput Electrode Biocompatibility Testing

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Introduction

Neural interfacing may be used to give patients with full or partial paralysis control over prosthetic devices or stimulate intact neural tracts below the point of injury. In some cases, electrodes are inserted into the central nervous system (CNS) to record signals from neurons of interest. A high failure rate is reported in literature with many electrodes losing signals within a few months (Moxon et al. 2009). Glial scarring is believed to be a major contributing factor to increased impedance and loss of signals during chronic tests (Polikov et al. 2005, Moxon et al. 2009).

To enable real-time visualization of the glial scarring process, an *in vitro* model supporting microglia and astrocyte (primary responders to foreign bodies in the CNS) is being designed. Various electrode design parameters will be tested in combination with animal studies to investigate glial scarring of microwires. In past work, 2D systems were insufficient due to electrode shifting and inability to model mechanical characteristics of the CNS, so a 3D model is being pursued.

This study proposes the use of a hyaluronan (HA) hydrogel for 3D culture of astrocytes and microglia. We believe a 3D culture model will alleviate issues associated with electrode shifting and better mimic the *in vivo* environment. Both cell viability and characterization are studied to evaluate the systems functionality as a model for foreign body response to Pt/Ir electrodes.

Materials and Methods

3D Cell Culture

Primary mixed glial cells were isolated from whole brains of day 1 Sprague-Dawley rats in accordance with University of Alberta animal ethics guidelines. HA was modified with methacrylic anhydride (MA, a functional group that can be polymerized) as shown previously (Smeds and Grinstaff 2001). Isolated mixed glial cells were then suspended in a 0.5 wt% methacrylated HA-MA precursor solution (0.1% w/v triethanolamine, 0.1% w/v 1-vinyl-2-pyrrolidinone and 0.01mM eosin Y). Radical polymerization was initiated with green LED light for 60 s.

Live/Dead Assay

Cell viability was investigated at various seeding densities (10^7, 50^7 and 10^8 cells/mL) and time points (1 and 7 days) using a membrane permeability assay. Syto13 labeled cell nuclei of all cells and Sytox Orange labeled cells with compromised plasma membranes. Gels were imaged with an inverted fluorescent microscope to determine viability.

Immunochemistry

Antibodies Iba1 (microglia) and GFAP (astrocytes) were used to label cell specific structures. Appropriate secondary antibodies conjugated to Alexa fluor 647 or Alexa fluor 488 were then used to visualize these cell structures with an inverted fluorescent microscope and Z-stacked imaged were compiled using ImageJ.

Results

Cell Viability

Preliminary results indicate that an increasing seeding density to 10^8 cells/mL results in increased viability upon encapsulation and sustained viability over 7 days (Figure 1). Cell count and attachment to the scaffold was also improved with increased seeding density (not shown).

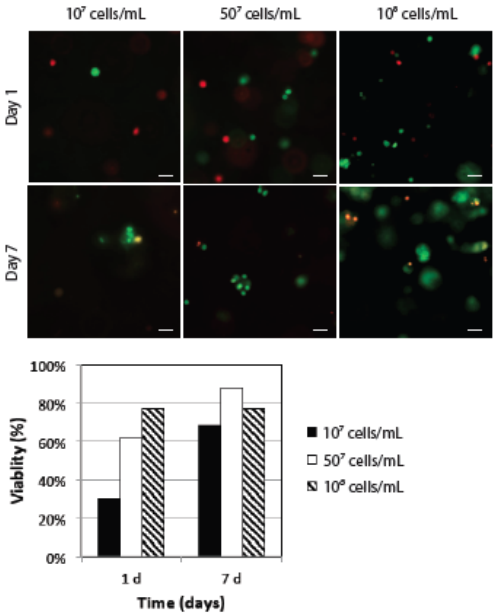

Figure 1: Preliminary results indicate increasing cell viability with increasing seeding density (n=2). Scale bar 20um.

Cell Characterization

The primary cell types of interest for investigation of the foreign body response are microglia and astrocytes. To verify the presence of these cell types within the HA-MA gel, antibodies against Iba1 (microglia) and GFAP (astrocytes) were used. Both astrocytes and microglia were present in the cells after 7 days of encapsulation. Astrocytes displayed signs of attachment and extended processes forming a network. Microglia were also present, but appeared to be in a spherical rather than ramified state (Figure 2).

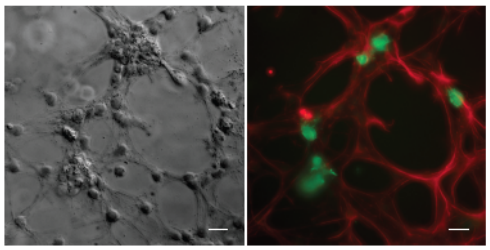

Figure 2: Iba1 (green) labeled microglia and GFAP (red) labeled astrocytes present in gel and extending processes after 1 week in culture. Scale bar 20 um.

Electrode stability was tested during culture (media changes) and labeling (multiple wash steps) by inserting an electrode (30um Pt/Ir) into the gel for 7 days. Both microglia and astrocytes were visible at the electrode interface (Figure 3) and minimal electrode shifting was observed

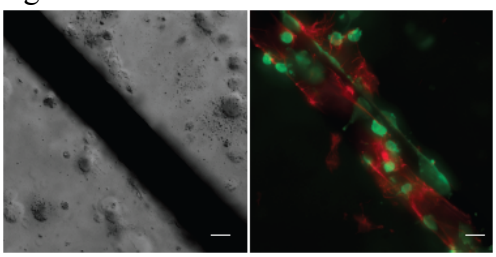

Fig. 3: Iba1 (green) labeled microglia and GFAP (red) labeled astrocytes line the Pr/Ir electrode after 1 week in culture. Scale bar 20 um.

Discussion

This study investigated the viability of mixed glial cells in a 3D scaffold over 7 days with varying seeding densities. Preliminary results indicated a trend of improved viability and attachment with increasing seeding density. This is most likely due to cell-cell signaling between glial cells. Further experiments must be done to determine the statistical significance of this improved viability. Immunochemistry was used to characterize cell types and their morphologies within the 3D HA-MA scaffold. Although astrocytes appeared to have a resting morphology with numerous interconnected processes, microglia were generally seen in their active state. Introduction of a Pt/Ir electrode did not interfere with cell characterization and the gel improved electrode stability during both culturing and immunolabeling. These results indicate that HA-MA is a promising scaffold for glial cells and would improve high-throughput testing of electrode biocompatibility. This improved model would not only reduce electrode shifting, but allow testing of the mechanical compatibility of the electrodes as well. Future work optimizing mechanical properties of the hydrogel (porosity and elastic modulus) is required to further improve the model.

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