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Living Electrodes: Tissue Engineering the Neural Interface

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***Abstract*— Soft, cell integrated electrode coatings are proposed to address the problem of scar tissue encapsulation of stimulating neuroprosthetics. The aim of these studies was to prove the concept and feasibility of integrating a cell loaded hydrogel with existing electrode coating technologies. Layered conductive hydrogel constructs are embedded with neural cells and shown to both support cell growth and maintain electroactivity. The safe charge injection limit of these electrodes was 8 times higher than conventional platinum (Pt) electrodes and the stiffness was four orders of magnitude lower than Pt. Future studies will determine the biological cues required to support stem cell differentiation from the electrode surface.**

I. Introduction

Bioelectrical interfaces are typically manufactured with electrically conductive materials that are biologically inert (e.g. gold, platinum, silicon) [1-3], however their stiff mechanical properties and smooth surfaces are not conducive to neural tissue integration [4, 5]. This leads to formation of a fluid gap between the electrode and surrounding tissue, resulting in scar tissue ingrowth. Ultimately, the bioelectrode becomes isolated leading to a need for more charge to be injected to achieve cell stimulation thresholds and a consequent higher power consumption of the device. To combat this well-known issue affecting all implantable electrodes, this work aims to investigate conductive, bioactive hydrogel electrode coatings embedded with cells to both reduce mechanical mismatch and improve native cellular integration between device and tissue.

Ideally, neural tissue should grow on the electrode surface to create an intimate junction between device and target. Numerous strategies to evade scar tissue infiltration have been proposed, including drug delivery, flexible electrodes, and conductive polymer (CP) coatings [3, 6]. CPs have gained popularity in bioelectrode coatings because of their biocompatibility, ease of tailoring, and ease of production, however they have poor mechanical properties. One promising method developed by Green et al. [7] combines the CP poly(ethylene dioxythiophene) (PEDOT) and a poly(vinyl alcohol) (PVA) hydrogel to create a hybrid material known as a conductive hydrogel (CH). The CH can be loaded with biological molecules to encourage cell growth towards the electrode and elute chemorepellents to reduce scar tissue formation while remaining electrically conductive. However, *in vivo* studies of CP and CH materials suggest that scar tissue remains a challenge in creating ideal electrode interfaces [8].

To address this issue it is proposed that the CH system can be expanded to enable a layer of encapsulated neural cells to be incorporated within the electrode coating. This new approach uses the knowledge from both bionic device and bioelectrode research combined with the principles of tissue engineering, which is more commonly used to replace bulk tissue types as a result of injury. This concept of integrating living cells with electrodes was first raised using a simple bacterial cell in 1980. Ochiai et al [9] first reported the term "living electrode" and proposed the use of live blue-green algae embedded in an alginate gel to act as an electric energy photoconverter. In a follow up study, Ochiai et al [10] noted that although stability of these biological electrodes was good, the power conversion efficiency was very low, i.e., ~0.1%. The latter limitation in efficiency has restricted further development of this concept.

In the biosensor field, Campbell et al. [11] embedded non-nucleated cells in the CP polypyrrole (PPy) electrodeposited onto metal electrodes. It was shown that cell surface antigen integrity was maintained, however as these are non-dividing, non-nucleated cells, and the application required only presentation of surface antigens, there was no requirement or investigation of long-term viability and cell function. More recently, Richardson-Burns et al [12] described electrodeposition of PEDOT on metal electrodes on which neuroblastoma-derived cell lines and primary mouse cortical cells were grown. Cells survived initial short-term exposure to monomers but apoptosis was observed at 72 hours with up to a third of cells being non-viable at 120 hours. Cytoskeletal disruption was also noted following the polymerisation of PEDOT, suggesting that focal adhesions may have been disrupted [12]. Despite subsequent research from the same group suggesting that PEDOT could be polymerised within living tissue [13], there has been little progress over the past 5 years on the concept of "living" electrodes with eukaryotic cells incorporated.

It is proposed that a layered system, such as that depicted in Fig 1, will address the problems with past designs. There are two layers depicted in this design, a CH layer and an overlying cellular layer. The CH is produced using established methods which have been shown to improve the charge transfer properties of Pt while providing a softer interface [7]. By encapsulating the cells in an overlying

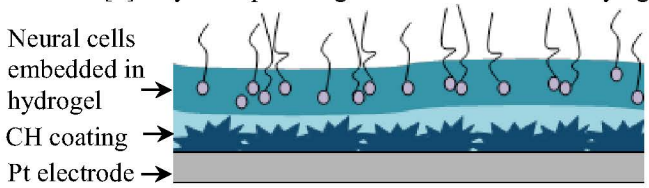

Figure 1. Schematic of layered "living electrode" design.

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hydrogel layer above the pre-formed CH coating they are protected from the solvents and high DC voltages required to produce the CH. The hydrogel used for this upper layer is a degradable hydrogel which provides biochemical and mechanical support for the neural cells, but also enables them space to grow and differentiate. As the hydrogel degrades, it is expected that the cells will generate their own matrix scaffold, effectively forming an integrated electrode interface with indistinct borders between the synthetic device and the surrounding tissue.

The first step in developing a living electrode is development of a hydrogel which can support survival and growth of the encapsulated neural cells. It is an additional requirement that this layer not inhibit the passage of charge from the underlying electrode surface. This paper presents the development of a bioactive degradable hydrogel based on PVA in which neural cells are encapsulated. This layer was formed by photopolymerization over a CH coated Pt electrode. The resulting construct was characterized electrically and mechanically in comparison to conventional Pt electrodes.

II. Methodology

A. Fabrication of living electrodes

For proof of concept, large Pt disc electrodes (10 mm diameter, 100 µm thick) were used. Pt discs were first deposited with a pre-coat of PEDOT. This adherence layer is produced by galvanostatic electropolymerisation from a monomer precursor solution consisting of 0.1 M EDOT and 0.05 M paratoluene sulfonate (pTS) in 50% DI water and 50% acetonitrile. Deposition was carried out at 1 mA/cm^2 for 1 min. The non-degradable hydrogel was then formed from a macromer solution of 18 wt% methacrylate modified PVA and 2 wt% methacrylate modified heparin (Hep-MA), using methods established by Nilsaroya et al. [14]. The hydrogel film was crosslinked to form a thin layer across the electrode surface with ultra-violet (UV) light (70 mW/cm^2, 365 nm) for 180s. PEDOT was deposited through this gel from an aqueous solution of 0.03 M EDOT at 0.5 mA/cm^2 for 30 min. The final layer was produced from a macromer solution of 9 wt% phenol modified PVA and 1 wt% sericin (extracted in-house from *Antheraea mylitta* silkworm cocoons.mylitta,) using methods developed by Lim et al. [15]. Neural cells (PC12, Marinpharm, Germany) were added to the macromer solution at 5 x 10^6 cells/ml. The initiators ruthenium (2mM) and sodium persulfate (SPS, 20mM) were included and a 15 µl drop of solution was placed on the electrode and a coverslip was used to create a thin uniform layer. The construct was exposed to visible light (15 mW/cm^2)for 180 s to crosslink the final hydrogel layer.

B. Neural cell survival and differentiation

To simplify imaging and allow time course analysis of cell growth, the cell loaded, degradable hydrogel described above was also produced as a standalone sample (without underlying CH or electrode). These samples were placed in an incubator with metal fences and cultured for 12 days at 37°C and 5% CO_2. The cells were maintained in Roswell Park Memorial Institute medium (RPMI-1640, Sigma-Aldrich, Aust.) supplemented with 10% horse serum (HS) and 5% fetal bovine serum (FBS). After 4 days, the medium was changed to promote differentiation by reduction of serum content to 1% HS and addition of 100 ng/ml nerve growth factor (NGF 2.51S, Jomar, Aust). At day 12 the cells were stained with calcein-AM and propidium iodide (PI) to enable visualization of live and dead cells, respectively, using fluorescent microscopy.

C. Electrical properties

Cyclic voltammetry (CV) was used to characterize the electrochemical activity of living electrodes at each stage of fabrication. A three electrode cell was formed with a large Pt counter electrodes and an isolated Ag/AgCl reference electrode. Voltage was cycled between -600 and 800 mV at 150 mV/s for 20 cycles using an eDaq potentiostat and eCorder unit (eDaq, Aust). The charge storage capacity (CSC) was calculated by integrating the resulting current waveform relative to time.

Charge injection limit was determined using protocols previously established by Cogan [16]. The limit was defined as the voltage required to reach the reduction potential for water. An in-house biphasic stimulator was used to deliver constant current, charge balanced pulses. The phase was incremented from 0.1 – 0.8 ms and the current was increased until the voltage reached –600 mV versus Ag/AgCl. The charge delivered in a single phase at this point was regarded as the charge injection limit.

Finally, frequency dependent impedance spectroscopy was determined using an eDaq impedance analyzer. The same 3 electrode cell was used to record the impedance of samples exposed to 50 mV sinusoids delivered from 1Hz to 100 kHz.

D. Mechanical properties

Finally, the stiffness of this composite coating was evaluated using peak-force quantitative nanomechanical mapping (PF-QNM) via atomic force microscopy (AFM). The Derjaguin-Muller-Toporov (DMT) model was used to calculate the stiffness modulus from hydrated samples under 1nN tapping forces applied at 0.5 Hz.

III. Results

A. Fabrication of living electrodes

The layered construct was produced and found to be robustly adherent to the underlying substrate. The CH was blue in color and the overlying cell loaded hydrogel was pale yellow from the ruthenium initiator. When soaked in media or saline for 12 hr the excess ruthenium was leached out and a slight brown color remained as evidence of the bound sericin proteins.

B. Neural cell survival and differentiation

Neural cells survived encapsulation and neural processes were extended at 12 days, as shown in Fig 2. Live-dead staining revealed that cell viability was excellent with no visible dead cells, which would present as red in the image. The degradable hydrogel remained intact and light microscope images revealed the polymer had developed a fibrous appearance. By comparing the fluorescent and phase contrast images it could be determined that the neural processes were aligned with polymeric stands, suggesting the cells were intimately bound through the polymer mesh.

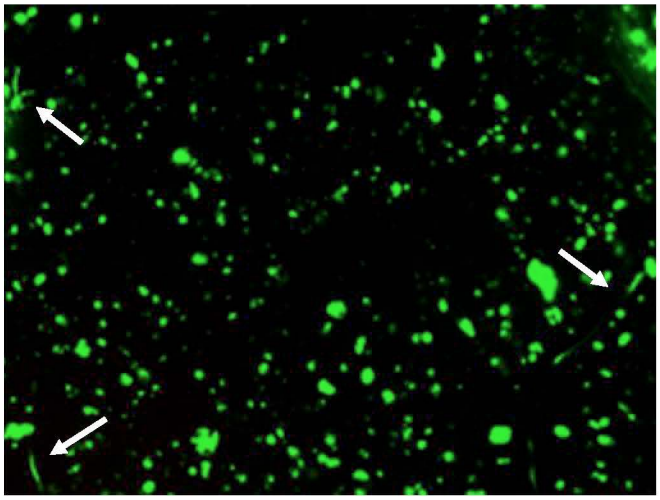

Figure 2. Encapsulated PC12s at 12 days show high viability and extension of neural processes (white arrows).

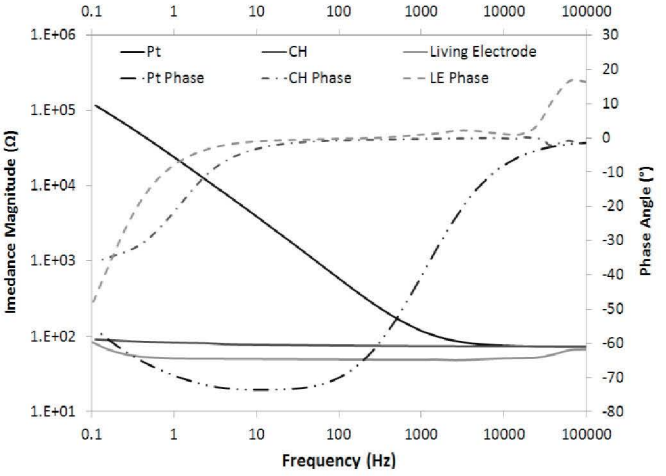

Figure 3. Electrochemical impedance spectroscopy bode plot of Pt, CH and living electrodes, N=3.

C. *Electrical properties*

The CSC of the electrodes was measured at each stage of fabrication, see Table 1. The CH coating was shown to increase the CSC of the underlying Pt by more than one order of magnitude. The subsequent layering with the degradable hydrogel increased this CSC two fold, to 50 mC/cm^2.

The injection limit of the living electrodes was also characterized using charge balanced biphasic waveforms. The cathodic injection limit for all materials is recorded in Table 1. The living electrode was found to have a higher injection limit than the Pt but was similar to the CH.

TABLE 1. CHARGE STORAGE CAPACITY AND INJECTION LIMIT OF ELECTRODES AT 0.2MS PHASE WIDTH (N=4)

Electrode	Charge Storage Capacity (mC/cm^2)	Cathodic Charge Injection Limit (mC/cm^2)
Pt	0.91 +/- 0.16	0.10 +/- 0.05
CH	24.96 +/- 3.96	0.81 +/- 0.07
Living electrode	50.00 +/- 8.71	0.83 +/- 0.12

The electrochemical impedance spectroscopy studies supported the voltammetry findings, which suggest an increased charge transfer capacity with addition of the degradable hydrogel layer. The impedance magnitude, shown in Fig. 3 for the living electrode was similar in magnitude to the CH and significantly lower than the Pt below 100 Hz. In the biologically relevant region of 100 – 1000 Hz the living electrode retained a lower impedance magnitude than both Pt and CH electrodes with a zero degree phase lag.

C. *Mechanical properties*

Modulus mapping revealed that the overlying degradable hydrogel was softer than both the underlying CH and the Pt electrode. The additional hydrogel layer reduced the modulus of the CH coated electrode by an additional order of magnitude, shown in Fig. 4.

IV. DISCUSSION

A living electrode was developed by producing a layered construct over conventional Pt electrodes. An initial layer of CH was used to improve the charge transfer properties and mechanics of the Pt. An overlying layer of degradable hydrogel which supports neural cell proliferation and differentiation was applied and found to further improve electrical and mechanical properties.

The encapsulated cells in the PVA-sericin hydrogel were found to have high viability following 12 days of culture. No dead cells were visualized and some neurite outgrowth was found. This suggests that the biosynthetic PVA/sericin hydrogel can provide sufficient biochemical support to maintain the PC12 cells during differentiation, which was promoted through NGF in the media. This concurs with findings by Lim et al. [17] who reported 1 wt% sericin/19wt% PVA hydrogels could promote adherence and proliferation of fibroblasts in a 2D culture. It was also found that this hydrogel system, which is fully degradable in 21 days when produced without cells [15], maintained its shape throughout the culture period. The presence of a fibrillar structure when viewed under light microscopy suggests that the cells bind with the sericin domains, preventing complete dissolution of the hydrogel structure. To successfully engineer a functional living electrode the degradable or partially degradable hydrogel must be tailored such that it degrades at a rate appropriate to matrix production. Although, cell support was confirmed, neurite outgrowth was low and

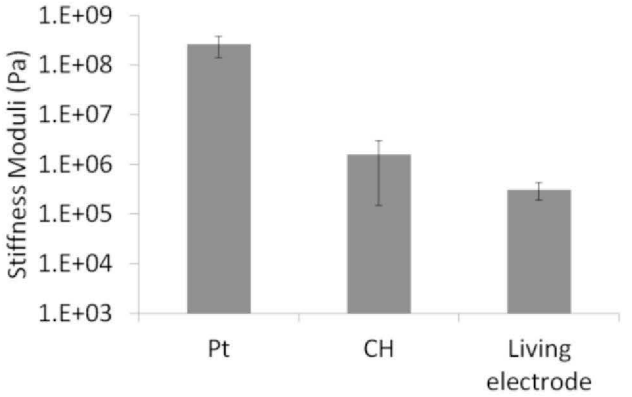

Figure 2. Stiffness modulus measured by peak-force nanomechanical mapping (N=3).

strategies for increasing differentiation need to be developed. PC12s, and many other neural cell types, do not produce their own matrix [18], as such future work will develop the support required for complex cells types and development of neural networks.

The additional hydrogel layer was found to improve the electrical properties of the coated electrode. While it is known that a CH produced with the conductive polymer PEDOT can significantly improve charge transfer [7], it was thought that the overlying polymer layer without an additional conductive component might restrict charge transfer. However, the living electrodes had increased CSC and lower impedance at low frequency than the CH coated electrodes. This is thought to be a result of the ionic partitioning properties of hydrogels as they hydrate and swell in an electrolyte [19]. The polar regions of the hydrogel polymer can sequester ions which can then be used to transfer charge on application of a stimulus. The accessibility of the aqueous electrolyte through the entire 3D structure of the hydrogel mesh, rather than just a 2D planar surface, such as that presented by a Pt electrode, increases the useable charge transfer area. However, the charge injection limit data did not show a significant difference between the CH and the living electrodes. It is believed that at short biphasic pulse widths (0.2 ms) the sequestered ionic charge is not fully utilized because longer time periods may be required to elute these ions through the thickness of the hydrogel layer. It is important to note, that while the cell integrated electrode coatings do improve the electroactivity of the electrode construct, conceptually in this design, only the encapsulated cells should be stimulated. Ideally, these encapsulated cells will be used for device communication with target neurons.

Finally, the mechanics of this layered system demonstrated a reduction in stiffness. It is generally accepted that interfacial stress and implant stiffness can increase scar tissue ingrowth rendering a neuroprosthetic implant nonoperational [1]. It has been proposed that coating of electrodes with various polymers can reduce interfacial stiffness. While Green et al. demonstrated that PEDOT and CH coating reduced Pt electrode stiffness, the coating remained several orders of magnitude stiffer than neural tissue [7]. The addition of a cell loaded hydrogel which can further dampen the mechanical mismatch between synthetic device and natural tissue is expected to be a significant advantage of this system.

V. Conclusion

Living electrodes are a new approach to tissue engineering the neural interface. By providing a "biological" layer between the electrode and the tissue, the living electrode has potential to mediate electrode tissue interactions by modifying mechanics and biological characteristics. The layered hydrogel construct provides a scaffold for supporting cell survival and differentiation without compromising the electrical performance of the electrode. This proof of concept study will provide the basis for a more complex system to support functional neural cells which can be tailored for different tissue types by manipulation of hydrogel chemistry. Ultimately, synapse formation will be an important next step to establishing the efficacy of this system.

References

[1] L. A. Geddes and R. Roeder, "Criteria for the selection of materials for implanted electrodes," *Annals of Biomedical Engineering,* vol. 31, pp. 879-890, 2003/07/01 2003.

[2] M. P. Ward, P. Rajdev, C. Ellison, and P. P. Irazoqui, "Toward a comparison of microelectrodes for acute and chronic recordings," *Brain Research,* vol. 1282, pp. 183-200, 7/28/ 2009.

[3] W. L. C. Rutten, "Selective electrical interfaces with the nervous system," *Ann Rev Biomed Eng,* vol. 4, pp. 407-452, 2002.

[4] R. A. Green, N. H. Lovell, G. G. Wallace, and L. A. Poole-Warren, "Conducting polymers for neural interfaces: Challenges in developing an effective long-term implant," *Biomaterials,* vol. 29, pp. 3393-3399, 2008.

[5] R. A. Green, S. Baek, L. A. Poole-Warren, and P. J. Martens, "Conducting polymer hydrogel composites for medical electrode applications," *Sci. Technol. Adv. Mater.,* vol. 11, pp. 1-13, 2010.

[6] C. E. Schmidt and J. B. Leach, "Neural tissue engineering: Strategies for repair and regeneration," *Annual Review of Biomedical Engineering,* vol. 5, pp. 293-347, 2003.

[7] R. A. Green, R. T. Hassarati, J. Goding, S. Baek, N. H. Lovell, P. J. Martens, and L. A. Poole-Warren, "Conductive hydrogels: Mechanically robust hybrids for use as biomaterials," *Macromol Biosci,* vol. 12, 2012.

[8] K. A. Ludwig, J. D. Uram, J. Yang, and D. C. Martin, "Chronic neural recordings using silicon microelectrode arrays electrochemically deposited with poly(3,4-ethylenedioxythiophene) (PEDOT) film," *J Neural Eng,* vol. 3, pp. 59-70, 2006.

[9] H. Ochiai, H. Shibata, Y. Sawa, and T. Katoh, "“Living electrode” as a long-lived photoconverter for biophotolysis of water," *Proceedings of the National Academy of Sciences,* vol. 77, pp. 2442-2444, 1980.

[10] H. Ochiai, H. Shibata, Y. Sawa, M. Shoga, and S. Ohta, "Properties of semiconductor electrodes coated with living films of cyanobacteria," *Applied Biochemistry and Biotechnology,* vol. 8, pp. 289-303, 1983.

[11] T. E. Campbell, A. J. Hodgson, and G. G. Wallace, "Incorporation of erythrocytes into polypyrrole to form the basis of a biosensor to screen for rhesus (d) blood groups and rhesus (d) antibodies," *Electroanalysis,* vol. 11, pp. 215-222, 1999.

[12] S. M. Richardson-Burns, J. L. Hendricks, B. Foster, L. K. Povlich, D.-H. Kim, and D. C. Martin, "Polymerization of the conducting polymer poly(3,4-ethylenedioxythiophene) (PEDOT) around living neural cells," *Biomaterials,* vol. 28, pp. 1539-1552, 3// 2007.

[13] M. R.-B. Sarah, L. H. Jeffrey, and C. M. David, "Electrochemical polymerization of conducting polymers in living neural tissue," *Journal of Neural Engineering,* vol. 4, p. L6, 2007.

[14] A. Nilasaroya, L. A. Poole-Warren, J. M. Whitelock, and P. Jo Martens, "Structural and functional characterisation of poly(vinyl alcohol) and heparin hydrogels," *Biomaterials,* vol. 29, pp. 4658-4664, 2008.

[15] K.S. Lim, M.-H. Alves, Y. Ramaswamy, L. Poole-Warren, P. Martens. "Covalent incorporation of unmodified, natrive proteins into synthetic hydrogels," *9th World Biomaterials Congress,* Chengdu, China, 2012.

[16] S. F. Cogan, P. R. Troyk, J. Ehrlich, and T. D. Plante, "In vitro comparison of the charge-injection limits of activated iridium oxide (AIROF) and platinum-iridium microelectrodes," *Biomedical Engineering, IEEE Transactions on,* vol. 52, pp. 1612-1614, 2005.

[17] K. S. Lim, J. Kundu, A. Reeves, L. A. Poole-Warren, S. C. Kundu, and P. J. Martens, "The influence of silkworm species on cellular interactions with novel PVA/silk sericin hydrogels," *Macromolecular Bioscience,* vol. 12, pp. 322-332, 2012.

[18] R. P. Bunge, "Expanding roles for the schwann cell: Ensheathment, myelination, trophism and regeneration," *Current Opinion in Neurobiology,* vol. 3, pp. 805-809, 10// 1993.

[19] S. Brahim and A. Guiseppi-Elie, "Electroconductive hydrogels: Electrical properties of polypyrrole-poly(HEMA) and electrochemical composites," *Electroanalysis,* vol. 17, pp. 556-570, Apr 2005.