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Surface immobilization of neural adhesion molecule L1 for improving the biocompatibility of chronic neural probes: *In vitro* characterization

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Abstract

Silicon-based implantable neural electrode arrays are known to experience failure during long-term recording, partially due to host tissue responses. Surface modification and immobilization of biomolecules may provide a means to improve their biocompatibility and integration within the host brain tissue. Previously, the laminin biomolecule or laminin fragments have been used to modify the neural probe's silicon surface to promote neuronal attachment and growth. Here we report the successful immobilization of the L1 biomolecule on a silicon surface. L1 is a neuronal adhesion molecule that can specifically promote neurite outgrowth and neuronal survival. Silane chemistry and the heterobifunctional coupling agent 4-maleimidobutyric acid *N*-hydroxysuccinimide ester (GMBS) were used to covalently bind these two biomolecules onto the surface of silicon dioxide wafers, which mimic the surface of silicon-based implantable neural probes. After covalent binding of the biomolecules, polyethylene glycol (PEG)–NH_2 was used to cap the unreacted GMBS groups. Surface immobilization was verified by goniometry, dual polarization interferometry, and immunostaining techniques. Primary murine neurons or astrocytes were used to evaluate the modified silicon surfaces. Both L1- and laminin-modified surfaces promoted neuronal attachment, while the L1-modified surface demonstrated significantly enhanced levels of neurite outgrowth ($p < 0.05$). In addition, the laminin-modified surface promoted astrocyte attachment, while the L1-modified surface showed significantly reduced levels of astrocyte attachment relative to the laminin-modified surface and other controls ($p < 0.05$). These results demonstrate the ability of the L1-immobilized surface to specifically promote neuronal growth and neurite extension, while inhibiting the attachment of astrocytes, one of the main cellular components of the glial sheath. Such unique properties present vast potentials to improve the biocompatibility and chronic recording performance of neural probes.

Keywords: Neural probes; L1; Laminin; PEG; Neurite outgrowth

1. Introduction

Silicon-based neural probes that permit recording and stimulation of specific sites in the brain experience failure during long-term recording, partly due to biocompatibility issues [1–4]. Their inability to chronically interface with the neurons in the brain is an immediate obstacle to their use in

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clinical applications, such as treatment of full or partial paralysis, which requires these implants to maintain a stable performance for the lifetime of the recipient.

Implanted electrodes record brain activity by detecting extracellular field potentials of neurons in reference to a ground. The closer the electrode is to a neuron, the better the signal strength and the quality of the recordings. To obtain reliable signals, the distance from microelectrode to neuron should be within 50–100 μm [5–7].

Immunohistological examinations have shown a significantly lowered neuronal density around the implant at different time points; this area is defined as the "kill zone" [4,5,8]. Several possible causes have been suggested. First, current implants are anchored to the skull at one end, and remain floating in the brain tissue at the end where the recording sites are located. Migration and micromotion of the implant occur, which may cause chronic irritation [9]. Second, the current implant material is not attractive to neuronal attachment and growth [10], therefore neuronal processes may tend to migrate away from the implant. Furthermore, neuronal death or degeneration may occur simply due to insertion injury or through factors that are released during chronic inflammatory responses [4].

Chronic gliosis results in glial sheath formation, which may encapsulate the probe and isolate it from the surrounding brain tissue. Gliosis is believed to be mediated by macrophages, activated microglia and reactive astrocytes. The reactive astrocytes are characterized by enhanced migration, proliferation, hypertrophy, upregulation of the glial fibrillary acidic protein (GFAP) and increased matrix production. Gliosis is hypothesized to cause significant impairment of implant functionality by increasing the electrode impedance, decreasing local neuron density and reducing axonal regeneration around the implant [4,5,7,11].

Several studies have addressed different methods of improving the performance of the chronic neuron–implant interface by modifying these implants using novel biomaterial designs. Some approaches use surface-immobilized cues to improve the attachment and growth of neurons, including electrochemical deposition of conducting polymers and neuron-promoting biomolecules on the electrodes [13–15], covalent immobilization of bioactive laminin-derived peptides on dextran-coated [16] and amino silane-modified silicon substrates [17], electrostatic layer-by-layer deposition of laminin [18], microcontact printing of poly-L-lysine [19], and electrospinning of silk-like polymer containing the laminin fragment IKVAV [20]. Soluble cues such as nerve growth factor and dexamethasone have also been incorporated for controlled release to promote neuronal ingrowth or reduce glial inflammation [21–23].

Many of the research efforts described above immobilize laminin or sequences of laminin onto the surface of neural probes for improved biocompatibility [13,14,16–18,20]. Laminin is an extracellular matrix protein (ECM) and a basement membrane component that has been shown to promote attachment of various cell types including neurons, astroglia, and fibroblasts [20,24–26]. This highlights the shortcoming of using non-neuron specific laminin on the surface of neural probes where an enhanced glial response could be undesirable [2,4]. L1 is a neural cell adhesion molecule, which is expressed in developing and differentiated neurons of the central nervous system (CNS) and Schwann cells of the peripheral nervous system (PNS) [27–29]. L1 mediates neuron–neuron adhesion via homophilic binding and has been shown to promote neuronal attachment and neurite outgrowth *in vitro* [30–33]. Neurite outgrowth, here is defined as the enhanced extension and growth of neurites once in contact with the L1 biomolecule present at the substrate. Unlike ECM proteins, L1 comes from the immunoglobulin family and is known to show tightly regulated patterns of expression during development of the nervous system, as well as selectivity in cellular binding partners [29,34]. When surface-bound to polystyrene substrates, human recombinant L1 supports significantly higher levels of neuron attachment and neurite outgrowth relative to the ECM protein fibronectin and poly-D-lysine, while inhibiting the attachment of astrocytes, meningeal cells and fibroblasts [33]. For these reasons, L1 was chosen as a candidate molecule to modify the silicon surface of neural probes to specifically promote neuronal attachment and inhibit glial cells. The long-term objective of this research is the improvement of neuron–electrode compatibility *in vivo* for further enhancement of long-term recording capabilities.

In this work, L1 is purified from murine brain tissue. To compare the efficacy of L1 and laminin as substrates for neuronal or astrocytic cell attachment and growth, these proteins were covalently immobilized on silicon dioxide wafers along with polyethylene glycol (PEG). Surface analysis, including water contact angle measurements, dual polarization interferometry (DPI) [35] and immunochemistry, were performed to characterize the modified silicon surfaces. Quantitative assessment of neuron and astrocyte attachment, and neurite outgrowth on the different silicon surfaces, was performed with primary neuron and astrocyte cultures and by using immunohistochemistry and fluorescent imaging techniques.

2. Materials and methods

2.1. Isolation and purification of L1 protein

The cell adhesion molecule L1 was purified by immunoaffinity chromatography as described by Lagenaur and Lemmon [30]. The L1 protein was affinity purified from detergent-containing extracts of crude membrane fractions of murine brains using 5H7 coupled monoclonal antibody packed columns. The membranes were solubilized in 1% 3((3-cholamidopropyl)dimethyl-ammonio)-propanesulfonic acid (CHAPS) (Anatrace), centrifuged at 40,000*g* for 30 min, and the supernatant was applied twice down the column. The antigen was eluted with 0.1 M diethylamine (Sigma–Aldrich) at pH 11.5 and immediately neutralized

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