Research Statement

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My research interests are focused on the use of tissue engineering approaches to restore the function of the nervous system. In the short term, my research areas will be focused on spinal cord repair, and neural prosthesis/brain interface using biomaterials, drug delivery, and stem cell therapy. In the future, the technologies developed in these studies will help extend my research into other areas in neural tissue engineering such as peripheral nerve regeneration, brain tumor therapy, retinal prosthesis, and regenerative electrodes,.

Project #1: Biomimetic scaffolds for stem cell–based therapy in spinal cord repair

Traumatic Spinal cord injury (SCI) causes neuronal loss, demyelination and/or transection of axons, and apoptosis of oligodendrocytes. Oligodendrocyte loss and demyelination are major secondary consequences of SCI, resulting in insecure impulse propagation or complete conduction failure[1,2]. In order to restore lost functions, injured neurons have to survive, and their axons need to regenerate across or around the lesion site and re-establish functional neural connections. The transected and injured axons need to become remyelinated in order to resume impulse propagation, which requires restoration of the oligodendrocyte population. However, axonal regeneration is restricted by the inhibitory environment in the CNS, and oligodendrocyte renewal is limited and insufficient to promote remyelination after SCI[1-3].

Stem cell therapy is a promising strategy to promote remyelination and functional recovery from SCI by replacing lost oligodendrocytes and neurons. We propose to develop *in situ* gelling hydrogel scaffolds for delivery of neural stem/precursor cells (NSPCs) and conformal repair of spinal cord defects. The scaffolds are functionalized with pro-regenerative extracellular matrix (ECM) protein laminin-1 (LN-1)[4-6] and growth factor sonic hedgehog (Shh)[7,8] to support NSPC adhesion, proliferation, and preferential differentiation into oligodendrocytes and neurons. We further propose to create a neurotrophin gradient in the scaffolds to promote and direct axon outgrowth. Agarose hydrogel scaffolds have been used to promote regeneration in both peripheral and central nervous system for the following appealing properties: (1) it is biocompatible and elicits only minimal inflammatory response when implanted in vivo; (2) it can be conjugated with bioactive molecules and still maintain its thermoreversible gelling property; and (3) it can be loaded with drug or gene delivery carriers for sustained release[9-11]. This proposal outlines the development and characterization of agarose-based biomimetic hydrogel scaffolds for enhanced spinal cord regeneration.

Phase I: In vitro development of an agarose scaffold with covalently coupled LN-1 and Shh

LN-1 and Shh will be bound to an agarose hydrogel scaffold to allow for NSPC attachment, expansion, and preferential differentiation. The gel scaffold will be mixed with NSPC culture system to assess the optimal concentration of bound LN-1 and Shh. NSPC survival, proliferation and differentiation will be characterized using live/dead assay, cell viability assay, and immunostaining.

Phase II: Generation of anisotropic agarose scaffolds with neurotrophin gradients in situ

Neurotrophin gradients function as neurotropic cues that guide and promote axon regeneration. However, anisotropic scaffolds are commonly pre-formed before implantation. Here we propose an innovative method to create neurotrophin (BDNF or NT3) gradients into the agarose scaffolds in situ. The concentration gradients will be quantified. Cortical neurons and dorsal root ganglia will be cultured in the anisotropic agarose scaffolds. Neurite outgrowth will be measured to assess the optimal neurotrophin gradients.

Phase III: In vivo analysis of biomimetic scaffolds in a rat spinal cord injury model

Once the agarose hydrogel systems are characterized, an agarose scaffold with each, or combination of the above therapeutic strategies, will be added to the injury site of a rat spinal cord injury model. Using immunohistochemistry, electron microscopy, electrophysiology, and behavior analysis outputs, each of the above strategies will be analyzed to assess their potential in promoting regeneration.

Project #2 Nanotechnologies for engineering neural prosthesis/brain interface

Stable single-unit recordings from the nervous system using microelectrode arrays can have significant implications for the treatment of a wide variety of sensory and movement disorders. However, the long-term performance of the implanted neural electrodes is compromised by the formation of glial scar around these devices, which is a typical consequence of the inflammatory tissue response in the CNS. The glial scar acts as a barrier that isolates neurons from the electrodes and raises the electrode impedance. The inflammatory tissue response results in neuronal degeneration, which compromises the functional state of the neuronal circuitry around the electrodes. To maintain long-term recording stability, the reactive gliosis and other inflammatory processes around the electrodes need to be minimized, and the damaged neurons and neural circuitry need to be restored[12,13].

My previous research shows that local release of anti-inflammatory agent from implanted neural electrodes attenuates inflammatory response and reduces neurite loss[14]. However, there is still a kill zone (a zone with significantly lower or non-existent neuronal density) around the implant site. This Therefore, a neurotrophic interface between the implanted neural prostheses and brain tissue is necessary for restoration of degenerated neurons and neural circuitry. We propose to modify the electrode surfaces with neurotrophic, anti-inflammatory coatings for enhanced tissue integration of neural prostheses and long-term recording stability. Two novel surface coating technologies, electrostatic layer-by-layer (LBL) self-assembly and matrix assisted pulsed laser evaporation (MAPLE), will be explored to generate functional coatings incorporating neurotrophins and anti-inflammatory agents. Both technologies allow for fine thickness control in nanoscale on substrates of various shapes and sizes, and preservation of the bioactivity of deposited biomolecules or drugs.

Phase I: Development of functional coatings capable of releasing neurotrophins and anti-inflammatory agents using electrostatic LBL assembly or MAPLE deposition

1. Electrostatic LBL assembly

Polyethyleneimine (PEI) is a highly positively charged polycation that readily attaches to oxidized surfaces including silicon dioxide (SiO_2), which is the major surface component of

many microelectrode array systems. Hence, in this study, PEI will be used to generate a positively charged layer on SiO_2 substrates, followed by alternate adsorption of negatively charged anti-inflammatory agents and positively charged neurotrophins to create coatings one nanoscale layer at a time. Control of LBL assembly and resultant structure will be based on Quartz crystal microbalance (QCM) analysis, ellipsometry, atomic force microscopy (AFM), scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FTIR). The release profiles of anti-inflammatory agents and neurotrophins will be characterized. In vitro cell culture studies will be used to determine the efficacy and optimal dose levels of anti-inflammatory agents and neurotrophins in the LBL assemblies. The effect of the nanoscale coatings on electrode impedance will be assessed using impedance spectroscopy.

2. MAPLE Deposition

Neurophins/anti-inflammatory agents alternating with biocompatible surface erosion polymers will be deposited on substrates using MAPLE technology, resulting in multilayer films that degrade under biological conditions to release one layer of drugs at a time. In collaboration with Dr. Roger Narayan at North Carolina State University, we have successfully deposited anti-inflammatory drug dexamethasone[4], and various biocompatible polymers on Si/SiO_2 substrates with this technology. Our preliminary data shows that dexamethasone released from poly (D, L) lactic/dexamethasone bilayer thin films remains bioactive. Release of bioactive molecules will be controlled by varying the number and thickness of drug and polymer layers. The coatings will be characterized using profilometry, AFM, SEM, FTIR, in vitro cell assay and impedance spectroscopy.

Phase II: In vivo evaluation of 'neurotrophic' and 'anti-inflammatory' potential of the coatings in a rodent model

Microelectrode arrays with the functional multilayer coatings will be implanted into the cortex of adult rats. Inflammatory response, neuron density, and neuronal circuitry around the electrodes will be assessed using immunohistochemistry, electron microscopy, and impedance spectroscopy. In vivo release of bioactive molecules will be characterized using fluorophore conjugation or radio labeling.

Phase III: In vivo evaluation of the performance of the optimized coating(s) in attaining long-term recording stability

The most promising coatings as evaluated in phase I and II will be applied to microelectrode arrays for evaluation of long-term functional recording stability in a rodent model. The change of the following parameters over time will be used to evaluate the recording stability of the implanted neural electrodes: number of active electrode sites, single unit stability, stability index, signal-to-noise ratio, recording longevity, and electrode impedance. If promising outcome of chronic recording is obtained in the rodent model, the coatings will be tested in a primate animal model.

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