SYMPOSIUM OO

Neurologic Biomaterials

November 29, 2000

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Symposium Support
Army Research Office
The Whitaker Foundation

**A joint Proceedings with symposium LL/MM/NN/OO
will be published as Volume 662
of the Materials Research Society
Symposium Proceedings Series.**

* Invited paper

This symposium endorsed by the Society for Biomaterials

SESSION OO1: NEUROLOGIC BIOMATERIALS I
Chairs: Christine E. Schmidt and Molly S. Shoichet
Wednesday Morning, November 29, 2000
Clarendon (Sheraton)

9:00 AM *OO1.1
BIOINTERACTIVE MATERIALS FOR ENGINEERING THE NEURAL INTERFACE. Patrick A. Tresco, University of Utah, The Keck Center for Tissue Engineering, Department of Bioengineering, Salt Lake City, UT.

Central nervous system (CNS) disorders represent a profoundly devastating set of health care problems. Many of which may possibly be repaired by biomaterial-based therapies that seek to restore function to the nervous system. The inability of transplanted or regenerating neurons to naturally grow over long distances through the damaged adult CNS perhaps may be overcome by the use of implanted materials that isolate, encourage, and direct axonal outgrowth to appropriate targets. A cornerstone of this engineering tour de force is the development of materials that are capable of providing the instructions needed to re-establish the functional architecture of the CNS. Toward this end, we have examined fundamental behaviors of primary CNS derived neurons, glia and fibroblastic cells cultured on surfaces of biomedically-relevant materials that differ in their physico-chemical properties. Our results indicate that material surface chemistry, microtopography, curvature, and the manner in which surface ligands are presented have profound effects on cell attachment, cell morphology, cytoskeletal structure, matrix organization and neurite outgrowth. In addition, our results indicate that the intrinsic properties of the cytoskeleton of certain CNS cells may be harnessed by engineered surfaces to direct the organization of apical adhesive ligands presented either on the cell surface or as a part of a secreted matrix that can be used to engineer the architecture of an adherent cell layer. These findings suggest ways of engineering biointeractive materials that possess living interfaces that serve guidepost functions, as occurs in early nervous system development; to direct the outgrowth of attached or regenerating neurons toward predetermined targets. Lastly, our findings suggest alternatives for engineering the interface of implanted biomaterials to encourage better integration with host neural tissue.

9:30 AM OO1.2
FUNCTIONAL RECOVERY FOLLOWING SPINAL CORD HEMISECTION MEDIATED BY A UNIQUE POLYMER SCAFFOLD SEEDED WITH NEURAL STEM CELLS. Erin Lavik, MIT, Dept. of MS&E, Cambridge MA; Yang Teng, Xian Lu Qu, Evan Snyder, Children's Hospital and Harvard Medical School, Dept. of Neurology, Boston, MA; Robert Langer, MIT, Dept. of Chemical Engineering, Cambridge, MA.

Traumatic spinal cord injury (SCI) includes axonal severance and the loss of neurons and glia. We have developed an implant to address the problems of cell death, axonal severance, and glial scar formation utilizing a unique biodegradable polymer scaffold fabricated from a blend of PLGA and a block copolymer of PLGA and polylysine and seeded with neural stem cells (NSCs). The seeded scaffold mimics the structure of a healthy spinal cord with an inner section seeded with the NSCs and an outer section with long oriented axial pores designed to facilitate axonal guidance coupled with radial pores to facilitate nutrient transport yet inhibit scar tissue formation. Female Sprague-Rats received a lateral hemisection of 4 mm the cord at the T9-T10 followed by implantation of the scaffold with cells, scaffold alone, cells alone, or lesion control. One day post injury and weekly thereafter, each animals functional recovery was rated via a series of behavioral tests including open-field walking, a host of reflex analyses, and inclined plane performance. Animals receiving scaffolds showed significant improvement in recovery of locomotion as well and reflexive behaviors with respect to the cells alone and lesion control groups. In particular, animals receiving scaffolds show weight bearing coordinated stepping in contrast to the control groups which showed no weight bearing and only some movement of the hindlimbs. Antegrade and retrograde tracing coupled with histological evaluation were performed to elucidate the role of the scaffold and cells in the recovery following traumatic injury.

9:45 AM OO1.3
RECONSTRUCTION OF THE INJURED SPINAL CORD USING NEUROGEL™ IMPLANT. Stéphane Woerly, Organoel Canada Ltée, Québec City, CANADA; Araceli Espinosa, Jean de Vellis, UCLA Mental Retardation Research Center, Los Angeles, CA; Jean D. Peduzzi, Christophere G. Paramore, Dept of Physiological Optics, Dept of Surgery, Division of Neurosurgery, University of Alabama at Birmingham, Birmingham, AL.

A hydrogel of poly [*N*-(2-hydroxypropyl) methacrylamide], Neurogel™, with a macro and mesoporous structure, a fractional porosity of 89%, and viscoelastic and diffusion properties similar to those of the neural tissue was developed as support matrix to promote tissue repair of the injured spinal cord (SC). Neurogel™ was implanted in the transected cat and in the chronically injured rat (compression) SC to promote the reconstruction of cellular components of the transection and syringomyelic cavity, and thereby establish a permissive environment for the growth of regenerating axons across the reconstructed lesion. Neurogel™ was implanted either immediately or three months after injury. In both models, the polymer implant bridged the transection site and the posttraumatic cavity with excellent integration with the host spinal tissue. Infiltration of astrocytic processes, mesenchymal cells, blood vessels within the pore spaces of the polymer gel resulted in the formation of a tissue that could fill up to 100% of the lesion. Double immunocytochemistry and confocal microscopy revealed considerable outgrowth of axons, mainly myelinated fascicles into the reconstructed lesion, and a significant reduction of the Wallerian degeneration of the white matter. Neurons immunopositive for neurofilaments were present in the gel implant containing the reparative tissue. Axonal labeling showed biocytin-stained descending axons that extended across the hydrogel into the distal segment of the spinal cord with formation of dendro-dentritic contacts. This finding was consistent with functional improvement that was observed compared to inured-only controls. This technology offers the potential to repair structurally lesion of the spinal cord that supports regenerative growth of axons and functional recovery.
Supported by Organogel Canada Ltée and the Spinal Cord Society.

10:30 AM OO1.4
A NEW SYNTHETIC PROCESS TO CREATE TUBES FOR USE IN SPINAL CORD INJURY REPAIR STRATEGIES. Paul Dalton, Molly Shoichet, Univ of Toronto, Dept of Chemical Engr & Applied Chemistry, Toronto, CANADA; Catherine Munro, Sunnybrook & Women's College Health Sciences Ctr, Div of Neurosurgery and Trauma Research Program, Toronto, CANADA; Ying Luo, Shaily Sanghvi, Univ of Toronto, Dept of Chemical Engr and Applied Chemistry, Toronto, CANADA; Eve Tsai, Univ of Toronto, Toronto Western Hospital Research Inst, Toronto, CANADA; Rajiv Midha, Sunnybrook & Women's College Health Sciences Ctr, Div of Neurosurgery and Trauma Research Program, Toronto, CANADA; Charles Tator, Univ of Toronto, Toronto Western Hospital Research Inst, Toronto, CANADA.

Spinal cord injury repair strategies have relied on cell transplantation, nerve autografts or synthetic hollow fiber membranes to provide a pathway to connect the severed ends; yet none are ideal. We are designing a multi-component tubular device that provides both haptotactic and chemotactic cues of regeneration. Herein we describe the new process to synthesize the tubes and preliminary in vivo results. Tubes were synthesized by adding a formulation (consisting of monomer, initiator, crosslinking agent and diluent) into a glass mold and then rotating the mold. Since only the monomer is soluble in the diluent, as polymerization proceeds, the polymer precipitates out of solution, and due to the centrifugal forces of rotation, the precipitated polymer forms a tubular structure. By controlling the formulation chemistry and the rotation speed, the properties of the resulting tubes are manipulated. Tubes have been prepared with 2-hydroxyethyl methacrylate (HEMA), methyl methacrylate (MMA) using a redox initiating system and at least 65 wt% water. The tubes have been characterized for: (1) morphology by an environmental scanning electron microscope (ESEM Model 2020); (2) tensile modulus by a micro-mechanical tensile tester (Dynatek Dalta); and (3) permability of B12, among other molecules, in a custom-built diffusion chamber. The tube wall structure can be tuned to have a range of morphologies from bicontinuous water/polymer phases to completely gel phases; similarly, the tube wall thickness can vary from 40 μm to 400 μm. Morphology and wall thickness affect the transport and mechanical properties. For B12, the diffusion coefficient varies from 10-7 to 10-9 cm^2/s while the modulus varies from 10 kPa to 3000 kPa. Ongoing in vivo studies are examining the tubes for their inherent strength and guidance potential in transection injuries in one of either the spinal cord or the sciatic nerve of adult rats.
Acknowledgment: We thank the Whitaker Foundation for partial financial support.

10:45 AM OO1.5
EMERGING PEPTIDE SCAFFOLD FOR NERVER TISSUE ENGINEERING. Todd C. Holmes*, Guosong Liu and Shuguang Zhang, Center for Biomedical Engineering, Departments of Biology, Brain and Cognitive Science, Center for Learning and Memory Massachusetts Institute of Technology, Cambridge, MA. *Department of Biology, New York University, New York, NY.

A new type of self-assembling peptide (sapeptide) scaffolds that serve as substrates for neurite outgrowth and synapse formation is described. This peptide-based scaffold is amenable to molecular design, using chemical or biotechnological syntheses. It can be tailored for a variety of applications. The sapeptide scaffold is formed through the spontaneous assembly of ionic self-complementary b-sheet oligopeptides under physiological conditions, producing a hydrogel material. The scaffold can support neuronal cell attachment, differentiation as well as extensive neurite outgrowth. Furthermore, it is a permissive substrate for functional synapse formation between the attached neurons. Since primary rat neurons form active synapses on such scaffold surfaces in situ, it suggests that they could be useful for tissue engineering applications. The buoyant sapeptide scaffolds with attached cells in culture can be readily transported from one environment to another. Furthermore these peptides did not elicit a measurable immune response, nor tissue inflammation when introduced into animals. These new biological materials created through molecular design and self-assembly may be developed as a biologically compatible scaffold for tissue repair and tissue engineering.

11:00 AM OO1.6
POLYPYRROLE-BASED BIOMATERIALS FOR NEURAL TISSUE ENGINEERING. Tyrell J. Rivers, Christine E. Schmidt, University of Texas at Austin, Department of Chemical Engineering, Austin, TX.

New tissue engineering technologies will rely increasingly more on interactive biomaterials that can both physically support tissue growth and stimulate specific cell functions without posing a health risk. In our past research, we have focused on biomaterials with electrical properties (i.e., the electrically conducting polymer, polypyrrole) which have been shown to improve the regeneration of several tissues including nerve and bone. In our recent work, we have modified polypyrrole for tissue engineering applications by either incorporating biological molecules which can specifically trigger desired cellular responses (e.g., the formation of new blood vessels), or by adding unique linkage sites within the polypyrrole backbone to control its degradation, mechanical integrity, and electrical properties. To this end, we have synthesized two distinct materials: (1) composites of polypyrrole and the polysaccharide hyaluronic acid which stimulates angiogenesis as it degrades; and (2) conducting pyrrole oligomers of three units in length connected using degradable ester linkages. Preliminary results suggest that polypyrrole/hyaluronic acid composites and biodegradable polypyrrole biomaterials are promising candidates for tissue engineering applications, such as nerve repair, that may benefit from electrical stimulation and/or enhanced vascularization.

11:15 AM OO1.7
E9 CHICK DORSAL ROOT GANGLION NEURITE EXTENSION DYNAMICS WITHIN THREE DIMENSIONAL AGAROSE GELS. Ryan Gilbert, Sebastian Corlette, Xiaojun Yu, Amit Balgude, Ravi Bellamkonda, Case Western Reserve University, Department of Biomedical Engineering, Cleveland, OH.

Understanding the differences in growth cone behavior within stimulatory (extracellular matrix) and inhibitory (glial scar) environments may allow for the development of materials that manipulate growth dynamics and facilitate central nervous system regeneration. Growth cones from E9 dorsal root ganglia were examined in three dimensional agarose gels, and gels coupled to either laminin (stimulatory environment) or chondroitin sulfate B (inhibitory environment). After 24 hours of incubation, growth cones were imaged every 60 seconds for a two hour period. Using image analysis software, neurite growth rates, time between consecutive growth phases (cycle times), and time spent in a particular part of the cycle were determined. Our data suggest that neurite extension is cyclical and that three sequential phases occur in each cycle. After the growth phase, neurites rest (resting phase), then search, displaying pseudopodia (searching phase), before growing once again. The time spent in a phase or cycle is a function of the chemical bound to the agarose backbone. Neurites grown in agarose-laminin gels spent more time in the growth phase than neurites grown in agarose gels. Additionally, neurites grown in agarose-chondroitin sulfate B gels spent more time in the searching phase than neurites grown in agarose gels. Cycle times were longer for neurites grown within agarose-chondroitin sulfate B gels and shorter for neurites grown within agarose-laminin gels compared to agarose gels. Such information, elucidating the dynamics of neurite extension, may assist in finding new neurite stimulating materials and may describe potential mechanisms for inhibition within the glial scar.
Acknowledgements: The Whitaker Foundation (RG98-0159).

11:30 AM OO1.8

NERVE GUIDE CONDUITS COMPOSED OF A BIODEGRADABLE POLY(PHOSPHOESTER): IN SITU DEGRADATION AND TISSUE REACTIONS. Shu Wang[1], Andrew C.A. Wan[1], Hai-Quan Mao[2], Hanry Yu[1], Kam W. Leong[2]. [1]Tissue Engineering Initiative, Institute of Materials Research & Engineering and National University of Singapore, SINGAPORE. [2]Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD.

In our previous studies, a poly(phosphoester), namely P(BHET-EOP/TC), has been successfully used as nerve guide conduits (NGCs) for reconstruction of 10mm gaps in a rat model. The polymer degrades in the presence of nucleophiles, with cleavage of phosphoester bond at random sites along the polymer chains. To further evaluate the potential benefit and risk of the polymer as a NGC material, its degradation rate and local tissue responses to its degradation products were studied. About 20% of weight loss was seen in our conduits after 12 weeks in PBS at 37°C. GPC analysis showed that the higher molecular weight fraction of the polymer had shifted to the lower end, confirming cleavage of polymer chains. The weight-average molecular weight had decreased from 15,000 to 10,000, a drop of 33%. Under scanning electron microscope, the surface of our NGC explants collected 3 months after implantation had changed from relatively smooth to very rough. GPC analysis of the explants showed a drop of 16% in the weight-average molecular weight, with a decrease in the polydispersity index from 2.22 to 1.76. This change, together with SEM pictures, confirmed that degradation of our NGC implants did occur in the body. DSC revealed the absence of any crystalline melting peaks for those materials in the range -20°C to 200°C, implying that crystallinity did not develop in a P(BHET-EOP/TC) NGC in vivo, at least up to a time period of 3 months. After in situ implantation in the sciatic nerve of the rat, the formation of a thin fibrous tissue capsule around the tube could be seen at 3 days. A well-organized fibrous tissue capsule was present on the outer surface of the conduit, with about 8 layers of fibroblasts and a thickness of about 20 mm. This low tissue reaction would be beneficial in minimizing adhesions of an implanted conduit to surrounding tissues.

SESSION OO2: NEUROLOGIC BIOMATERIALS II
Chairs: Ravi V. Bellamkonda and Molly S. Shoichet
Wednesday Afternoon, November 29, 2000
Clarendon (Sheraton)

2:00 PM *OO2.1
PROTEIN AND CELL DELIVERY SYSTEMS FOR THE BRAIN. W. Mark Saltzman, School of Chemical Engineering, Cornell University, Ithaca, NY.

Growth factors execute critical functions during the formation of specialized tissues throughout the developing embryo. When growth factors are provided to adult animals, they often encourage regeneration or repair of organs damaged by disease or trauma. But most growth factors have short half-lives after intravenous injection, with their biological activity lasting only a few min. Some promising new methods for growth factor delivery involve polymers, which can be engineered to provide precisely controlled, prolonged growth factor delivery at a localized site. In this presentation, new methods of growth factor delivery are illustrated using nerve growth factor (NGF) delivery to the nervous system as an example. To evaluate the biological activity of the NGF, and to illustrate the potential utility of this approach for tissue engineering, delivery systems were tested for their ability to enhance survival and function of transplanted cells.

2:30 PM OO2.2
SOL-GEL OPTICAL SENSORS FOR GLUTAMATE. Jenna L. Cox, UCLA, Neuroengineering, Los Angeles, CA; Esther Lan, UCLA, Department of MS&, Los Angeles, CA; Allan J. Tobin, UCLA, Brain Research Institute, Los Angeles, CA; Jeffery I. Zink, UCLA, Department of Biochemistry and Chemistry; Bruce Dunn, UCLA, Department of MS&E, Los Angeles, CA.

The amino acid glutamate is the major excitatory neurotransmitter used by the nervous system for inter-neuronal communication. It is used throughout the brain by various neuronal pathways including those involved in learning and memory, locomotion, and sensory perception. Because glutamate is released from neurons on a millisecond time scale into micron dimension spaces, the development of a glutamate biosensor with high temporal and spatial resolution is of great interest for the study of many major disease models. This work demonstrates feasibility of the development of an optic fiber glutamate sensor that takes advantage of sol-gel methods to encapsulate the enzyme glutamate dehydrogenase (GDH) on the fiber tip. Sol-gel materials containing the GDH and its co-factor, NAD, were produced by acid hydrolysis of tetramethyl orthosilicate (TMOS) followed by pH controlled condensation. Upon the addition of glutamate at varying concentrations (10μM 1mM), the enzymatic reaction was monitored via the production of NADH by both absorbance (340nm) and fluorescence (470nm). At saturated NAD concentrations, the initial reaction rate varied linearly with the log of

the glutamate concentration, and the reaction reached equilibrium conditions that were predicted by the K_{eq} from the literature. GDH does not leach from the gel upon extended soaking in buffer. Thin films containing GDH were also produced. The enzyme remained active in the film, and the reaction was monitored via fluorescence. These results indicate that the sol gel encapsulation of glutamate dehydrogenase results in an active enzyme with the expected behavior and whose reaction rate can be used to predict glutamate concentration within the physiological range.

2:45 PM OO2.3
STUDY OF THE ROLE OF PROTEIN-LIPID INTERACTIONS IN HEALTHY AND DISEASED MODEL MYELIN MEMBRANES USING THE SURFACE FORCES APPARATUS. Yufang Hu, Ben Ohler, Cindy Husted[1], Jacob Israelachvili Department of Chemical Engineering, University of California at Santa Barbara, Santa Barbara, CA. [1]Neuroscience Research Institute, University of California at Santa Barbara.

The stability of the myelin membrane structure is a key to the understanding of Multiple Sclerosis (MS), which is believed to be an auto-immune disease characterized by the demyelination of the neuron axons. Currently the cause and effective treatment of MS remain unknown. In this ongoing study, we take a physical chemical approach to study the behavior of a model membrane which is compositionally similar to the native myelin membrane. Our focus is the role of lipid/protein interactions on the adhesion between myelin membranes. The adhesion behavior of this model membrane is tested using a number of techniques including optical video microscopy, dynamic light scattering, freeze fracture microscopy, and surface force measurements. The first half of the project involves the interactions between lipid bilayers without myelin proteins. Our results indicate that the bilayer-bilayer interactions are dominated by molecular packing and electrostatics. Next, a myelin native protein, Myelin Basic Protein (MBP) is introduced into the model bilayer system. The emphasis of the second half of the project is on the role of MBP on bilayer adhesion. In particular, we are interested at the factors that modulate the degree of inter-bilayer adhesion. Our primary candidates are lipid composition, lipid packing, solution ionic strength, and temperature. We hope that the results from this study will provide insights into the possible cause of myelination/demyelination processes.

3:30 PM *OO2.4
SUSTAINED LOCALIZED DELIVERY OF CHEMOTHERAPEUTICS FROM MICROSPHERES IN ANIMAL MODELS OF GLIOMA: PRINICPLES FOR EFFECTIVE CNS DELIVERY. Dwaine F. Emerich, Raymond T. Bartus. Alkermes, Inc., Cambridge, MA.

These studies examined the ability of PLG microspheres, formulated to release carboplatin for 2-3 weeks, to prolong survival in a rodent model of glioma. Rat glioma cells were implanted into the cortex of rats and allowed to grow for 10 days prior to surgical resection. Rats were given either surgical resection only, bolus injection (100 ug) or microspheres containing 10, 50 or 100 ug of carboplatin. The microspheres were implanted, via hypodermic injection, either directly into the surgical cavity or into the tissue along the perimeter of the cavity. The order of survival among treatment groups was: no resection ¡ resection only < bolus chemotherapy < sustained release chemotherapy. The enhanced survival with sustained release was dose-related and substantially greater when the microspheres were implanted into the perimeter wall of the resection cavity, compared to implantation into the cavity itself. The enhanced survival produced by implants along the resection perimeter was associated with a significant attenuation of regrowth of the tumor. Atomic absorption spectrophotometry revealed that while the microspheres produced significantly prolonged tissue levels of carboplatin relative to a bolus injection, carboplatin diffusion was limited to brain tissue primarily 0.5 mm from the injection site. Data will also be presented examining the proportion of the tumor perimeter that requires exposure to carboplatin, and the how the distribution of micropsheres within the tumor perimeter effect efficacy. Together, these data: (1) support the continued development of biodegradable microspheres as a means to deliver chemotherapeutic agents to brain tumors, and (2) suggest that successful implementation of this approach in humans may require measures that overcome or improve upon restricted spatial drug diffusion from the microspheres.

4:00 PM OO2.5

SURFACE BOUND MOLECULAR GRADIENTS FOR DIRECTING NEURAL CELL GROWTH. Richard A. Gemeinhart, W. Mark Saltzman, Cornell Univ, School of Chemical Engineering, Ithaca, NY.

The development of neurological tissue has been proposed to follow gradients of repulsive and attractive cues. These cues are present both as diffusing substances and as surface bound substances. In the past, researchers have produced surface gradients of molecules by various means, but the production of a known gradient (shape or surface density) on a surface was not possible. For this reason, the study of neural development in vitro has yet to unequivocally prove the role of surface bound gradients of growth cues. In this work, the groundwork was laid for a system by which the two dimensional patterning of molecules can be combined with the production of surface gradients of molecules within the patterns. By using a photoactive poly(ethylene glycol), glass surfaces have been modified to be cell resistant in specific patterns. This criteria is necessary to deduce the specific reaction to the patterned surfaces. By addition of known concentrations of fluorescent labeled photoactive poly(ethylene glycol), fluorescent labeled surfaces were created at an intensity that reflected the concentration of fluorescent labeled poly(ethylene glycol) added, indicating a similarity of reactivity of the fluorescent, photoactive poly(ethylene glycol) and the photoactive poly(ethylene glycol). By exposure to light at the appropriate wavelength for specific amounts of time, the photoactive, fluorescent poly(ethylene glycol) was attached to the surface with intensities corresponding to the exposure time. In future studies, neurotrophins will be substituted for the fluorescent dye and cellular response will be examined on these surfaces. The successful production of a gradient of neurotrophin will allow the growth of neurons in a specific location and in a specific direction. Devices could then be produced to promote neural regeneration. These same devices could be used to study the development of neural tissue in vitro.