SYMPOSIUM MM

Cardiovascular Biomaterials

November 28, 2000

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SESSION MM1: CARDIOVASCULAR BIOMATERIALS I
Chairs: Jennifer L. West and Robert M. Nerem
Tuesday Morning, November 28, 2000
Clarendon (Sheraton)

8:30 AM MM1.1
PROMOTING THE HEMOCOMPATIBILITY OF SYNTHETIC BIOMATERIALS BY ENGINEERING SURFACES AND MODULATING FORCES. Diego Mantovani, Gaétan Laroche, Group of Interdisciplinary Research on Bioengineering, Dept of Materials Engineering and Surgery, Quebec, QC, CANADA.

It is estimated that 500,000 prosthetic bypasses are performed annually worldwide, in both thoracic and peripheral vascular surgery. While the use of saphenous vein grafts represents the primary surgical choice for lower limb vascular surgery, it can be used only in up to 30% of the patients, principally in reason of its poor availability in diseased patients. While, small diameter arterial prostheses (internal diameter less than 8 mm) made of expanded PolyTetraFluoroEthylene (ePTFE) are among the most widely used synthetic substitutes in peripheral vascular surgery, it has been shown by several works that the 4 years primary patency rate with ePTFE prostheses is only 12% for bypasses below the knee. Despite several industrial and/or academic projects has been carried out in the past to develop a synthetic surface showing blood compatibility, only a surface showing more or less anti-thrombogenic properties has been proposed. We believe that the endothelial cell coverage is the key parameter to develop an hemocompatible polymer-based surface. Our aim is to study the potential to develop a nano-engineered synthetic surface on which endothelial cells can successfully adhere, spread, and proliferate under a controlled mechanical environment. Low pressure plasma surface treatments are advantageous techniques for the design and development of new biocompatible materials because surface modifications can be achieved without altering the material bulk properties. In this context, surface modifications of ePTFE vascular prostheses by plasma treatment offer a promising way to achieve significant hemocompatibility improvements (1), particularly in the monolayer chemical nano-lithography context. In recent years, new evidence has come to light which indicates that the mechanics may control the biology of cells, how they control their membrane ion channels, their cytoplasmic actin, and the genes they express. Thus, in the particular case of the cardiovascular system, it is probable that mechanical forces acting on the arterial wall may control the mechanisms by which cells adhere, spread and proliferate onto all arterial prosthesis surfaces (2).
REFERENCES
1. D Mantovani et all., Mater Res Soc Symp Proc., 1999,544,21-26.
2. C.S. Chen et all.. Science, 1997,276,1425-1428.

8:45 AM MM1.2
MUCIN COATING ON POLYURETHANE IMPLANTS FOR BETTER BIOCOMPATIBILITY. Lei Shi and Karin D. Caldwell, University of Utah, Center for Biopolymers at Interfaces, Salt Lake City, UT; Gregory Burns, Utah Artificial Heart Institute, Salt Lake City, UT.

Previous study of mucin as a biological surfactant used for biomaterials shows that mucin coating could greatly change the surface hydrophobicity of polymer materials, thus, to form a non adhesive surfaces on the materials. In this work, mucin coated polyurethane material, a material used for artificial heart and vascular devices, was implanted into a sheep. Fibrinogen and Pluronic® F108 coated same materials were the comparisons. The host responses were examined by histologic evaluation. It has been found that mucin coated materials caused the most benign inflammatory response with a thickness of 0.03 mm for the formed capsule, which is significantly less than that produced by the other coated materials. The protein uptakes on these implants were analyzed by amino acid analysis and showed that, after 30 days in vivo, the protein surface concentrations were 15, 20, and 27 mg/m^2 for mucin, F108 and fibrinogen coated surfaces respectively. Mucin coating on the polyurethane material proved to establish a more inert surface for host tissue compared to the F108 coating, showing that this biological surfactant is superior to the synthetic surfactant in long-term biocompatibility.

9:00 AM MM1.3
HEMOCOMPATIBILITY OF MICROSYSTEMS MATERIALS. Shuvo Roy and Aaron Fleischman, The Cleveland Clinic Foundation, Department of Biomedical Engineering Cleveland, OH.

The development of microsystems for biomedical applications (BioMEMS) promises to revolutionize the field of patient diagnostics and therapeutics through the development of miniature components with sophisticated functionality that will enable seamless interaction at the interface of biology and engineering. BioMEMS components have been proposed for a variety of cardiovascular applications including microsurgical instruments, implantable sensors and implantable drug delivery systems. However, a search of the peer-reviewed literature indicates that the available information on the hemocompatibility of typical structural materials for microsystems is quite limited, thereby hindering device design and leading to extended product development and regulatory cycles. Accordingly, we have conducted investigations into the hemocompatibility of common BioMEMS materials - silicon, silicon dioxide, and silicon nitride - using test protocols based on the ISO Biocompatibility Guideline. Tests are conducted with human blood components to determine the influence of BioMEMS materials on hemolysis, osmotic fragility, prothrombin time (PT), and partial thromboplastin time (PTT). The paper will present details on materials preparation, test protocols, and the corresponding hemocompatibility results.

9:15 AM MM1.4
EFFECTS OF COMPOSITION ON SWELLING AND DEGRADATIVE CHARACTERISTICS OF INJECTABLE HYDROGELS USEFUL FOR CARDIOVASCULAR APPLICATIONS. Albert K. Shung, Antonios G. Mikos, Rice University, Department of Bioengineering, Houston, TX.

The overall goal of this project is to develop an in-situ polymerizable, biodegradable material for use in cardiovascular applications that minimizes non-specific cell adhesion and contains functional moieties for the attachment of peptides to induce specific cell attachment. A novel copolymer has been developed incorporating poly(propylene fumarate) (PPF) and poly(ethylene glycol) (PEG) which satisfies these criteria. PPF is a new biodegradable polymer currently being investigated for orthopedic and cardiovascular applications while PEG is a hydrophilic polymer that has been extensively studied for biomedical applications. The copolymer is chemically crosslinked with PEG diacrylate using an ammonium persulfate-ascorbic acid redox initiator system to form the hydrogel. The PEG and PPF block lengths can be varied to modulate the properties of the hydrogel formed. In this study, the PPF and PEG block lengths and the initial water content were varied to examine their effects on swelling, degradation and crosslinking density. A factorial experimental design was implemented to assess which of these three parameters had the greatest impact on swelling, degradation and crosslinking density. Swelling was found to be most affected by the initial water content followed by PEG block length and PPF block length. The swelling of the hydrogels ranged from 48% water uptake with low initial water content to up to 77% water uptake with the high initial water content. After three weeks, degradation of the hydrogels ranged from 4-13% mass fraction lost. Crosslinking density was determined by measuring the tensile strength of the various hydrogel formulations.

10:00 AM *MM1.5
PLATELET, LEUKOCYTE AND COMPLEMENT ACTIVATION BY CARDIOVASCULAR BIOMATERIALS. Cynthia H. Gemmell, Maud B. Gorbet and Michael V. Sefton, Institute of Biomaterials and Biomedical Engineering University of Toronto, Toronto, Ontario, CANADA.

Nonthrombogenicity of biomaterials is characterised conventionally by a long clotting time and a low cellular (platelet) deposition. These appear to be inadequate. Platelet deposition can be minimized by using hydrogel coatings, but platelet consumption and by extension microemboli formation are enhanced. Although platelets make transient contact with hydrogels (and other materials), they do not stick. Rather they are activated and preferentially removed from the circulation. Controlling platelet consumption, may be harder than simply controlling deposition, since the two are in part inversely related. We focus now on understanding the mechanism of biomaterial associated platelet activation and the companion processes of biomaterial associated complement and leukocyte activation. The latter is also of interest, since activated monocytes contribute to thrombin generation through tissue factor.

10:45 AM MM1.6
THE LIFE INITIATIVE: A MULTI-CENTER COLLABORATION TO ADDRESS THE VITAL ORGAN SHORTAGE. M.V. Sefton Inst of Biomaterials and Biomedical Engineering, Univ of Toronto, Toronto, Ontario, CANADA.

The Life Initiative began in Toronto on June 2, 1998 with the goal of using tissue engineering to create an essentially unlimited supply of vital organs (heart, kidney, liver) for transplantation. The medical capacity to treat disease is frustrated by the limited availability of donated organs. There are 150,000 patients world-wide who are

waiting for a transplant and demand for transplants is growing at the rate of 15%/year. Many more could benefit from a transplant were there an unlimited supply of organs and no waiting lists for a transplant. With an unlimited supply of vital organs, replacing a damaged or failed organ becomes not substantially different than getting 'a part changed in one's car'. One approach would be to grow human heart muscle cells outside the body, in a scaffold made from a degradable material in the shape of the heart. The resulting heart muscle together with separately prepared valves and other components would be assembled into the final product. The organs would then be stored 'in a box' until needed by the surgeon. Donor waiting lists would become a relic of the past. The LIFE initiative is an international collaboration of scientists, engineers and clinicians whose goal is to achieve this vision within a decade.

11:00 AM MM1.7
AMMONIA RF-PLASMA ON PTFE SURFACES: CHEMICAL CHARACTERIZATION OF THE SPECIES CREATED ON THE SURFACE BY VAPOR - PHASE CHEMICAL DERIVATIZATION. Pascale Chevallier, Martin Castonguay, Diego Mantovani, Gaetan Laroche, Quebec Biomaterials Institute and Laval University, Department of Mines, Metallurgy and Materials, Quebec City, QC, CANADA.

A cylindrically-configured low pressure plasma treatment system for Radio Frequency Glow Discharges (RFGD) fed with ammonia was developed to modify the internal surface of ePTFE arterial prostheses, without altering the material bulk properties. Reactive functional groups such as amines were introduced on the surface by the use of NH3 plasmas. Therefore, biomolecules with appropriate hemocompatibility properties could be solidly grafted on the surface (covalent attachment) through a reaction with the amine groups. In this context, the control of subsequent biomolecule grafting on the surface requires characterization of the surface chemical composition after ammonia RF plasma treatment. Indeed, initial XPS (X-Ray Photoelectron Spectroscopy) analyses clearly indicate that 15-20% of N were grafted onto the surface. However, amine moieties are not the sole chemical species formed onto the surface as defluorination is higher than expected. Moreover, positive identification of N-containing species by High Resolution XPS is often difficult owing to the small chemical shift differences and therefore the overlap of the XPS features of the different N-species. To overcome this problem, chemical derivatization was performed by exposing the plasma-treated surfaces to chemicals that react specifically with one or the other of the moieties created onto the surface in the plasma environment. In order to allow easy detection through XPS, the chemicals that were selected bore an atom that was not previously present neither on the surface nor created by the plasma treatment. Vapor-phase derivatization was privileged to eliminate the problem of surface rearrangements associated with solution-phase derivatization. The results have shown that surface derivatization is a powerful tool that allows an accurate identification of the surface species, therefore enabling the modulation of the surface characteristics through control over the plasma parameters.

11:15 AM MM1.8
Abstract Withdrawn.

SESSION MM2: CARDIOVASCULAR BIOMATERIALS II
Chairs: Wenda C. Carlyle and Dennis Goupil
Tuesday Afternoon, November 28, 2000
Clarendon (Sheraton)

1:30 PM MM2.1
A CELL-SEEDED COLLAGEN-GEL MODEL FOR THE STUDY OF VASCULAR CELL BIOLOGY. Barbara Imberti, Stephanie Kladakis, Dror Seliktar, Robert M. Nerem, Parker H. Petit, Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA.

Tissue-engineered blood vessel substitutes have the potential not only to serve as a vascular graft, but also to be used as a model system for the study of vascular endothelial cell (EC) and smooth muscle cell (SMC) biology. The system developed in the Georgia Tech/Emory Center for the Engineering of Living Tissues is one of in which human SMC are incorporated into a reconsituted type I collagen gel to form a tubular construct, followed by the seeding of human EC on the lumenal surface. This model system can be employed in either a tubular or slab configuration. Furthermore, the tubular constructs, once fabricated, can be used to study the effects of cyclic stretch and/or those due to exporsure to laminar shear stress. Initial studies have focused on the effects on SMC of mechanical conditioning, i.e. cyclic stretch, and after four days of mechanical onditioning constructs exhibited circumferentially oriented collagen fibrils and SMCs. Furthermore, such cyclic stretch resulted in an enhancement of SMC production of matrix metalloproteinase-2, i.e. MMP-2. More recent studies have focused on EC function using constructs which have been mechanically conditioned and then, following EC seeding, exposed to a laminar shear stress of 10 dynes/cm^2. These studies indicate the the reduction in EC proliferation due to fluid shear stress is influenced by whether or not the underlying substitute has been mechanically conditioned. These are now being extended to include investigations of EC migration; however, from results obtained to date it is concluded that the structural characteristics of the underlying wall of construct have an important influece on EC biology.

1:45 PM MM2.2
CARDIOVASCULAR TISSUE ENGINEERING THROUGH MICROFLUIDIC NETWORKS WITH ENDOTHELIAL AND STEM CELL SYSTEMS. Philip LeDuc, Amy Brock, Children's Hospital/Harvard Medical School, Dept of Pathology, Boston, MA; Shuichi Takayma, George M. Whitesides, Harvard Univ, Dept of Chemistry and Chemical Biology, Cambridge, MA; Donald E. Ingber, Children's Hospital/Harvard Medical School, Depts of Pathology and Surgery, Boston, MA.

The development of novel cardiovascular engineering constructs requires the creation of small diameter vascular networks with complex architectures that will support vascular growth and development. Although exciting progress has been made by modulating pulsatile flow, the application of this technology to smaller systems is in its infancy. By utilizing novel photolithographic techniques, microfluidic branching devices can be created on the micrometer scale. These devices support the attachment, growth and proliferation of capillary endothelial cells within a defined geometric region. With this synthetic and controlled technique, we can create complex branching architectures that simulate the vasculature. In addition, this method permits the creation of a two-cell, layered system such as the endothelial-smooth muscle cell layers found in the vasculature. We demonstrate that this system can be exploited to control localized differentiation of human mesenchymal stem cells. Undifferentiated stem cells are seeded in the microfluidic device on a surface coated with extracellular matrix protein. Soluble growth factors are delivered to stem cells via the microfluidic system and stimulate the differentiation of this single cell type into two different adjacent cell layers. Because the flow is laminar there is no mixing between the lanes of growth factors. Using this method, two cells in close proximity receive completely different chemical signals and respond by activating distinct genetic programs, mimicking the process of development in vivo. This two-cell system provides the future possibility of introducing a single cell type into a vascular graft and then producing a compatible two layer system through localized growth factor stimulation.

2:00 PM MM2.3
IN VITRO EVALUATION OF BIODEGRADABLE POLYMER SCAFFOLDS FOR VASCULAR TISSUE ENGINEERING. B.A. Nasseri, I. Pomerantseva, U.A. Stock, D.P. Martin, J.G. Lien, S.N. Oesterle, J.P. Vacanti, Department of Surgery, Cardiology Division, Massachusetts General Hospital, Boston, MA and the Center for Innovative Minimally Invasive Therapies, Boston, MA.

Objectives: Three different biodegradable polymer scaffolds intended for small caliber vessel tissue engineering were evaluated for seeding efficiency of vascular smooth muscle cells (SMCs) and endothelial cells (ECs). Materials and Methods: Tubes with 5 mm internal diameter and 20 mm length (n=8) were constructed from a) polyglycolic acid (PGA) felt sheet (1mm thick, 60mg/cc density), b) 5% poly-4-hydroxybutyrate (P4HB) foam sheet (1mm thick, made by freeze drying), and c) a combination of the above polymers (PGA mesh dipped in 1% P4HB solution). Tubes were coated with collagen solution (Vitrogen) for 4 hrs in 37°C and placed in suspension of ovine vascular SMCs (11x106 cells/tube). Tubes were incubated in a rotating bioreactor (5 rpm) in cell suspension (DMEM high glucose supplemented with 10% fetal calf serum, 1% Glutamine-Penicillin-Streptomycin and 2ng/ml basic human fibroblast growth factor) for 48 hrs. Thereafter, medium was changed daily. Cell count was performed from the medium 24 hours after seeding. Samples from all tubes were obtained for H&E and scanning electron microscopy (SEM) analysis after 24 hrs and after 7 days. On day 8 medium was replaced with suspension of ovine vascular ECs (0.6x106 cells/tube). Cell count from the medium was performed 24 hrs later. All tubes were fixed for H&E and SEM evaluation on day 14. Results: Cell count performed from the medium 24 hrs after SMC seeding showed that 6%, 7% and 24% of seeded cells remained in the medium with PGA, combination and P4HB tubes respectively. Cell count performed 24 hrs after EC seeding showed that 14%, 9.5% and 29% of seeded cells remained in the medium, suggesting better cell attachment to PGA and combination materials than to P4HB foam. After 7 days, H&E and SEM evaluation indicated that all surfaces of PGA and composite tubes were covered with several layers of confluent cells,

while P4HB did not show complete coverage and displayed some bare spots. Isolated dead cells were noted within P4HB polymer and multiple live spindle-shaped cells were observed infiltrating PGA and composite constructs. Similar observations were seen after day 14. PGA fiber degradation was observable within 24 hrs of exposure to medium. P4HB and composite tubes maintained tubular shape throughout the experiment; PGA tubes did not. Conclusions: Three-dimensional biodegradable tubes made from PGA felt coated with 1% P4HB solution show superior cell attachment and mechanical stability compared to tubes constructed from PGA or P4HB alone.

2:15 PM MM2.4
USING INDENTATION AND INTRA-VASCULAR ULTRASOUND TO MEASURE ARTERIAL RESPONSE. Peter M. Anderson, Eric Glaser, Alexander I. Veress, George Pharr, Geoff Vince and J. Frederick Cornhill, Ohio State University, Dept. of MS&E, Columbus, OH; Guidant Corportation; University of Utah; Oak Ridge National Laboratory; Cleveland Clinic Foundation.

Two alternatives to standard tensile testing of arteries are discussed. The first involves inflation of arteries and simultaneous measurement of arterial wall displacements with intra-vascular ultrasound (IVUS). The second involves the measurement of load versus displacement during microindentation of the intimal surface. The IVUS technique offers the potential for in-vivo measurement of mechanical response and the indentation technique offers the potential to measure mechanical inhomogenities in the vicinity of an atherosclerotic plaque, for example. The IVUS technique is used to study the nonlinear stiffening of porcine coronaries during inflation. Processing of the IVUS data relies on accurate determination of the intimal surface and medial/adventitia boundary during inflation. The microindentation technique is used to study the effect of rate of loading on tissue stiffness, recovery, and internal dissipation. Processing of the data requires accurate determination of the initial contact point between the indenter and intima. In summary, both techniques appear to successfully capture the significant nonlinear, time-dependent properties of arterial tissue. The presentation will conclude with challenges to refine each of these techniques.

3:00 PM MM2.5
NITRIC OXIDE PRODUCING HYDROGELS REDUCE SMOOTH MUSCLE CELL PROLIFERATION AND PLATELET ADHESION. Kristyn S. Bohl, Jennifer L. West, Dept. of Bioengineering, Rice University, Houston, TX.

Photopolymerizable derivatives of polyethylene glycol (PEG) have been synthesized that contain groups that hydrolyze under physiological conditions to produce nitric oxide (NO). Such groups are called NO donors and include moieties such as S-nitrosothiols and diaziniumdiolates. By covalently garfting different NO donors into photopolymerized PEG hydrogels, we have developed materials that produce NO for periods ranging from hours to months, depending on the NO donor group utilized. Materials can be formed from blends of polymers with different NO donor groups to generate multi-phasic release profiles. Thin hydrogel coatings can be formed on arterial surfaces via interfacial photopolymerization. These coatings can then serve as localized NO therapy to prevent thrombosis and restenosis after cardiovascular procedures such as angioplasty. We have demonstrated that these materials can halt smooth muscle cell proliferation without reducing cell viability and that exposure to the NO-producing hydrogels prevents platelet adhesion to collagen surfaces.

3:15 PM MM2.6
DESIGN OF pH SENSITIVE MATERIALS FOR ON/OFF RELEASE OF THROMBOLYTIC AND ANTICOAGULANT DRUGS. Angela M. Thornton and Christopher S. Brazel, University of Alabama, Dept of Chemical Engineering, Tuscaloosa, AL.

An experimental study was conducted to determine the mechanisms of transport for delivery of cardiovascular agents using a pH-sensitive hydrogel as the carrier. Copolymer gels based on hydrophilic (2-hydroxyethyl methacrylate) and polybasic (N,N-diethylaminoethyl methacrylate) monomers were formed as membranes and analyzed for their potential to control the diffusion of heparin and streptokinase. The polybasic materials were selected because they would allow drug delivery to be modulated by microenvironmental pH fluctuations around the site of a blood clot. Diffusion of model solutes, as well as heparin and streptokinase, were studied to determine the influence of gel morphology and mesh size on the screening of the solutes. In slightly basic solutions, the polymers remained in a thermodynamic state of phase-separation (polymer fraction ¿ 0.8), while the polymer absorbed more solution and the mesh size increased once the pH was less than the pKb of the polybasic moiety. Hydrogels representing a range of comonomer compositions were tested, with equilibrium swelling ratios determined as a function of pH. The permeability of the hydrogels to streptokinase was studied using side-by-side diffusion cells and compared to release profiles for the same drug loaded into the polymer by equilibrium partitioning. Results confirm that the large size of streptokinase restricts free diffusion in the polymer membrane. Streptokinase release studies conducted at both pH 2.2 and pH 8 indicate slow release profiles dependent upon the polymer swelling ratio. However, a significant portion of the release occurs as a burst, indicating that the loading is non-uniform, most likely through surface adsorption and interpenetration of the long protein chain into the hydrogel mesh. To achieve rapid release of these large molecular weight drugs, either high swelling or macroporous gels are required.

3:30 PM MM2.7
NEW RADIOACTIVE STENTS - A GENERAL APPROACH VIA NANOPOROUS COATINGS. Thomas Sawitowski, Wolfgang Brandau, Alfons Fischer, Guenter Schmid, Univ of Essen, Essen, GERMANY.

PTCA is a well established method to open up stenotic coronary vessels. In combination with stent implantation problems related for example to the elastic recoil or the vessel after PTCA can be reduced. The major drawback of the PTCA with stent implantation is the fact that in 30 - 40% of all interventions a restenosis of the vessel occurs. This restenosis is caused by several factors like for example the damage to the vessel wall by the stent and the balloon or by heavy metal ions dissolved by corrosive processes from the stent itself. A very promising way to prevent restenosis is the local brachytherapy. By a local irradiation of the insured vessel wall the cells stop to proliferate in a pathogenically way. In principal there are two irradiation devices used up to now for brachtherapy. The first is the stent itself, the second is a radioactive catheter. In both systems mainly different types of -emitters are used. Here we report on the development of a new kind of stent coating which enables us to irreversible bind different radioactive isotopes like $^{186/188}$Re, ^{99m}Tc, ^{103}Pd, .. with activities up to 200 MBq/stent. This coating is made of an amorphous ceramic which consists of very small pores in the nanometer region. These pores have diameters between 5 and 15 nm depending on the fabrication process. In combination with a chemical surface modification of the inner pore walls the isotopes bind covalently to the pore insides. Due to the small pore size almost no enzyme and no cell can enter the pore. For these reasons the binding of the isotopes is irreversible even under biologically conditions. We will report on the coating itself as well as on in vitro testing of the activated stents. Also the result of first animal trials performed with nanoporous coated commercially available 316L stainless steel stents will be presented.

3:45 PM *MM2.8
E. Edelman, Massachusetts Inst of Technology, Cambridge, MA.

ABSTRACT NOT AVAILABLE