SYMPOSIUM MM
Cardiovascular Biomaterials

November 28, 2000

Chairs

Jennifer L. West
Dept of Bioengineering
Rice Univ
MS-142
Houston, TX 77251-1892
713-348-5955

Wenda C. Carlyle
St. Jude Medical
Petaluma, CA 94952
707-762-8309

Robert Nerem
Petit Inst for Bioengr & Biosci
Georgia Inst of Technology
Rm 300
Atlanta, GA 30332-0363
404-894-2768

Dennis Goupil
BioCure Inc
Norcross, GA 30071
678-966-3422

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 MM1: CARDIOVASCULAR BIOMATERIALS

Chairs: Jennifer L. West and Robert M. Nerem
Tuesday Morning, November 28, 2000
Cherndon (Sheraton)

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

It is estimated that 596,000 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 patients, principally in cases of poor availability of diseased patients. While, small diameter arterial prostheses (internal diameter less than 8 mm) made of expanded PolyTetrafluoroEthylene (e-PTFE) are among the most widely used synthetic substitutes in peripheral vascular surgery, it has been shown by several works that the 4-year primary patency rate with e-PTFE prostheses is only 12% for bypasses below the knee. Despite several industrial and/or academic projects and a growing interest in the past to develop a synthetic surface with low 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 new-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 bio-compatible materials because surface modifications can be achieved without altering the material bulk properties. In this context, surface modifications of e-PTFE 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 act on the arterial wall may control the mechanics by which cells adhere, spread and proliferate on all arterial prosthetic surfaces (2).

REFERENCES

8:45 AM MM1.2
MUCIN COATING ON POLYURETHANE IMPLANTS FOR BETTER HEMOCOMPATIBILITY, Lei Shi and Kwan Y. Goh, University of Utah, Bioengineering 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 comparison. 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 the three materials were analyzed by protein acid and showed that, after 30 days in vivo, the protein surface concentrations were 15, 20, and 27 mg/m2 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, Shave Roy and Aaron Fleschman, 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 sophistication 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 devices, 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 limited, thereby hindering the development of 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, clotting factor, procoagulant time (PT), partial thromboplastin time (PTT). The paper will present detailed results, 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. Shang, Antonio G. Mikes, Rice University, Department of Biotechnology, Houston, TX.

The overall goal of this project is to develop 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 novel biocompatible polymer being investigated for orthopedic and cardiovascular applications whereas PEG is a hydrophilic polymer that has been extensively studied for biomedical applications. The copolymer is chemically crosslinked with PEG diisocyanate using an ammonium persulfate-acetic 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 43% 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. Gemmill, Michelle Miser, Gertot and Michael Selan, University of British Columbia and Biomedical Engineering University of Toronto, Toronto, Ontario, CANADA.

Nonthrombogenicity of biomaterials is characterized 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 microembolic 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 interdependent. 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, IV. Selent et al., 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 viable organs (heart, kidney, liver) for transplantation. The capacity to treat disease is frustrated by the limited availability of donor organs. There are 150,000 patients worldwide 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 not an unlimited supply of organs. With a limited supply of 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 placed into the body, and the muscles would be activated 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-PLASMA ON PTFE SURFACES: CHEMICAL CHARACTERIZATION OF THE SPECIES CHEATED ON THE SURFACE BY VAPOR PHASE CHEMICAL DERIVATIZATION. Pascal Chevallier, Martin Casteignoux, Diego Mostovian, Gaetan Laroché, Quebec Biomatériaux and Énergie, Quebec City, QC, CANADA.
A cylindrically-confined 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 plasma. Thus, biomolecules with appropriate hemocompatibility properties could be covalently grafted to the surface (covalent attachment) through a reaction with the amine groups. In this context, the control of subsequent biocidal grafting on the surface requires a precise knowledge of the chemical composition of the surface 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 dehydroamination is higher than expected. Moreover, positive identification of N-containing species by High Resolution XPS is often difficult to the small chemical shift differences and therefore the overlap of the XPS features of the different 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 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; Shashi Takayama, George M. Whitesides, Harvard Univ, Dept of Chemistry and Chemical Engineering, Cambridge, MA; Director. Douglas Singer, Children’s Hospital/Harvard Medical School, Dept 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 microimeter scale. These devices support the attachment, growth, and propagation of capillary endothelial cells within this microvascular network region. With a combination of controlled techniques and co-culture, 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 cells layers found in the vasculature. We have shown that the system can be exploited to control localized differentiation of human mesenchymal stem cells. Unseeded stem cells are seeded in the microfluidic device on a surface coated with extracellular matrix protein. Stable 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 layers 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. Novak, L. Ponsanenew, U.A. Stock, D.P. Martin, J.G. Cotten, J.P. Oesterle, J.P. Vasconi, 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: Polyethylene glycol (PEG) scaffolds, 5 mm in diameter and 20 mm length (n=8) were constructed from α,β polyglycolic acid (PGA) felt sheet (1mm thick, 60mg/cm² density), b) 5% poly-L-hydroxybutyrate (P4HB) foam sheet (1mm thick, made by freeze drying), and c) a combination of the above polymers (PGA mesh, mixed in 1% P4HB solution). Tubes were coated with collagen solution (Vitrogen) for 4 hrs in 37°C placed in suspension of ovine vascular SMCs (1x10⁶ cells/tube). Tubes were incubated in a roller bioreactor (1rpm) in cell suspension in cell culture medium supplemented with 10% fetal calf serum, 1% Glutamine, Penicillin-Streptomycin and 2ng/ml basic human fibroblast growth factor (24 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 ECs (1x10⁶ cells/tube). Cell count performed from 24 hrs after suspension showed that 0%, 7% and 34% of seeded cells remained in the medium with PEG, 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. At day 7, H&E and SEM evaluation indicated that all surfaces of PEG foam and composite tubes were covered with several layers of confluent cells, with 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 the EC function using constructs that have been mechanically conditioned and then following EC seeding, exposed to a laminar shear stress of 10 dynes/cm². These studies showed the enhancement in EC proliferation due to fluid shear stress is influenced by whether or not the underlying substrate 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 characterization of the underlying wall of construct has an important influence on EC biology.
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 observed within 24 hrs of exposure to medium. P4HB and composite tubes maintained tubular shape throughout the study. PGA tubes did not. Conclusions: Three-dimensional biodegradable tubes made from PGA felt coated with 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 MUSCLE RESPONSE: Patrick A. Lappin, Eric Glaser, Alexander I. Veraas, George Pharr, Geoff Vince and J. Frederick Cornhill, Ohio State University, Dept. of MS&E, Columbus, OH; Guidant Corporation; 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 intravascular 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 homogeneities in the vicinity of an atherosclerotic plaque, for example. The IVUS technique is used to study the nonlinear hardening of porcine coronary arteries during inflation. Processing of the IVUS data results on accurate determination of the arterial 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 diazomonoamines. By covalently grafting different NO donors into photopolymerizable 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-phase 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 ANTI-COAGULANT DRUGS: Angela M. Thornton and Christopher S. Brazil, 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 (3-hydroxypropyl methylacrylate) and polyvinylidene (N,N-dimethylacrylamide) monomers were formed as membranes and analyzed for their potential to control the diffusion of heparin and streptokinase. The polymeric materials were selected because they would allow drug delivery to be modulated by intravascular 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, pH 7.4, 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 pKa of the polymeric moiety. Hydrogels representing a range of monomer 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

3:30 PM MM2.7 NEW RADIOACTIVE SENTS - A GENERAL APPROACH VIA NANOPOROUS COATINGS: Thomas Kannanz, Wolfgang Brand, Alfonso 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 125I/111In, 90Y, 131I, 186Re, 99Mo/99mTc, 131I, with activities up to 200 MBq/uptake. 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.