Wednesday Afternoon, November 6, 2002

Plasma Science
Room: C-103 - Session PS+BI-WeA

Plasma Processing for Biocompatible Surfaces
Moderator: H.J. Griesser, University of South Australia

2:00pm **PS+BI-WeA1 Application of Plasma in Tissue Engineering**, ***R.D. Short***, *D.B. Haddow, S. MacNeil, R.A. Dawson, D. Barton, S. Fraser*, University of Sheffield, UK **INVITED**

A novel device which comprises an acrylic acid plasma polymerized carrier substrate, which supports the attachment and release of human keratinocytes, has been used to successfully treat non-healing (chronic) skin wounds. In proof of concept studies, weekly delivery of keratinocytes, initially obtained from a small 2x1cm biopsy and expanded up many hundred fold, have promoted healing in diabetic foot ulcers and other indications. In this paper we explain the rationale behind this therapy and review the results (to date) from the treatment of the first seven patients. Although the "mode of action" of the device is still to be fully understood, the role the plasma polymer plays in promoting cell attachment and detachment is key to the success of the device. The physical and chemical nature of the plasma polymer has been explored in some detail, and to a first approximation, we are able to describe the features of the plasma polymer that promote cell attachment and speculate on why cells transfer to an in vitro human dermal wound bed model. By means of a multi-technique approach (mass spectrometry,quartz crystal microbalance, ion flux probe, x-ray photoelectron spectroscopy, secondary ion mass spectrometry) we have begun to unravel the processes by which the acrylic acid plasma polymer grows. A case is made for better understanding of plasma polymer growth mechanisms, rather than treating the plasma polymerization process as a "black box" that produces materials with desired properties.

2:40pm **PS+BI-WeA3 Can Plasma Polymerised Surfaces Promote the Co-culture of Human Dermal Fibroblasts and Human Epidermal Keratinocytes in the Tissue Engineering of Skin?**, ***M.C. Higham***, *S. MacNeil, R.D. Short*, University of Sheffield, UK

Within the field of tissue engineering there is a need to develop new approaches to achieve effective wound closure in patients with extensive skin loss or chronic ulcers. Plasma polymers are synthetic surfaces capable of influencing and controlling cell physiology either directly or through an adsorbed protein layer. This project exploits the well-known interdependency of epithelial keratinocytes and stromal fibroblasts in conjunction with plasma surface technology. The aim of my project is to produce a chemically defined surface, which with the aid of a feeder layer of lethally irradiated dermal fibroblasts will improve the performance of the keratinocyte cell. Unable to divide yet remain physiologically active, irradiated fibroblasts aid keratinocyte attachment and proliferation from which sub-confluent cells can be transferred to wound bed models. Plasma co-polymers of acrylic acid/octa-1,7-diene have been prepared and characterised using X-ray photon spectroscopy (XPS). The fibroblasts and keratinocytes were cultured on plasma polymer coated 24 well plates. Cell attachment and proliferation were assessed using MTT-ESTA and DNA assays. The performance of both cell types on the plasma polymer surfaces was compared to Tissue Culture Plastic (TCPS) and Collagen I, plus a negative control of a pure hydrocarbon layer. A pure acrylic acid surface, fabricated at a power of 10W and containing 9% carboxylate group was found to promote both fibroblast and keratinocyte attachment and proliferation and permit the co-culture of keratinocytes with irradiated fibroblasts. The performance of this surface was comparable to collagen I, a well-established substratum for the attachment of keratinocytes. Current work is examining the potential of plasma polymer surfaces within the field of tissue engineering for transfer of keratinocytes onto an in vitro wound bed model and thereafter clinical trials.

3:00pm **PS+BI-WeA4 The Role of Reactive Neutral and Ionic Species in the Deposition of Organic Thin Films from an Isopropyl Alcohol and Argon Plasma**, ***D.C. Guerin***, National Research Council, Canada, *V.A. Shamamian*, Naval Research Laboratory

We present the measurements of neutral species in an argon/isopropyl alcohol (iPrOH) plasma, using appearance potential mass spectrometry. IPrOH is a potential precursor for the cost-effective plasma deposition of non-fouling surfaces. This work complements previous research on the ionic character of the plasma. It had been discovered that tuning the plasma pressure and power caused large variations in the dominant ionic reactions. The resulting changes in the chemical nature of the ionic flux were reflected in the functional character of the deposited films. A significant flux of neutral radicals was detected at the deposition surface at low plasma pressure. However, at higher pressures the plasma region was more remote and the neutral radicals were completely attenuated. The attenuation mechanism was determined to be reaction with the precursor. For example, the methyl radical abstracts hydrogen from iPrOH. Thus, as the pressure increases, the methyl radical flux evolves into a flux of methane. Mean free path (MFP) calculations for hydrogen abstraction agree with the experimental results. At low pressures, the reactive MFP is larger than the chamber geometry. At higher pressures, the reactive MFP is much smaller than the distance between the plasma and deposition surface. The ability of the reactive ions to diffuse from the remote plasma to the deposition surface is explained as being due to charge exchange limitations. The radical species generated have lower ionization energies than iPrOH or argon. Thus, the radical ions are energetically unable to react with the main species in the plasma. In contrast, the flux of ions with ionization energy greater than that of iPrOH, such as argon and methane, is highly attenuated at higher pressures. These results provide some context to competing claims as to the importance of neutrals and ions in deposition from molecular plasmas.

3:20pm **PS+BI-WeA5 Plasma Micropatterning for the Spatially Controlled Adsorption of Proteins**, ***J.D. Whittle***, *R.D. Short, D. Barton, A.G. Shard*, University of Sheffield, UK

Many biological interactions are surface mediated, for example protein adsorption and subsequent cell adhesion. In vitro it may be desirable in a number of applications to exert spatial control over these interactions. i.e. Limiting the attachment of cells to particular surface regions. We investigate the use of masks as a method of fabricating surfaces with patterned chemistry by plasma polymerisation, with feature sizes down to around 10µm. We utilise imaging secondary ion mass spectrometry (SIMS) and fluorescent light microscopy to visualise these chemical patterns. We also show how these chemical patterns affect the adsorption of proteins, not only in terms of the the amount of adsorbed protein, but also their conformation. A natural extension of depositing well-defined regions of chemistry (patterns) is to be able to fabricate regions of controlled chemical change (gradients), the properties of which vary continuously along the length of the deposited feature without any sharp transitions. We show how plasma polymerisation may be used to deposit chemical gradient surfaces with chosen endpoints (for example, a gradient running from a hydroxyl though to an amine dominated surfaces), and profile (for example, linear, sigmoidal etc.) by careful manipulation of the plasma composition and deposition surface during the treatment. These gradient surfaces can be used to examine the affect of changing a particular surface parameter (for example, the surface concentration of amine functionalities) on protein adsorption.

3:40pm **PS+BI-WeA6 Chemical Surface Micropatterning by Plasma and VUV Photochemical Modification of Polymers for Controlled Cell Culture.**, ***N.A. Bullett***, *F.E. Truica-Marasescu, M.R. Wertheimer*, Ecole Polytechnique, Canada

The three dimensional nature of the biomolecular environment in contact with cells has an important influence on the initiation and control of cell processes such as adhesion, migration, growth, protein secretion and gene expression. Traditionally, cell culture uses homogeneous substrates with no control over the biochemical and topological features in the immediate vicinity of the cells. The shape of mammalian cells is determined by the interaction of cell contact receptors with other cells or extracellular matrix proteins. Regulation of the shape of cells may enhance the function and differentiation of the cells. Surface modification of polymeric materials by low-pressure plasma and VUV photochemical treatment provides a convenient route to the fabrication of well defined chemically functionalised surfaces. A variety of functional groups may be introduced into the polymer surface, including amine and hydroxyl. Using these techniques it is possible to engineer surfaces that have a wide variety of applications in biomaterials technology, such as cell and protein adhesive surfaces or non-fouling surfaces. Complex micropatterns of chemically different regions have been produced by the selective functionalisation of the polymer using photolithographically defined masks. By this method, chemically distinct regions are produced at the micrometer scale, with a third dimension being provided by nanoscale topographical features. This three dimensional environment, on the nano- or micrometer scale, provides a complex but controllable surface for the culture of many different cell types. Characterisation of the micropatterned surfaces has been performed by XPS, FTIR, imaging TOF-SIMS and fluorescence microscopy. The surfaces have subsequently been used to study the attachment and growth of various cell types, for example bone-derived cells with orthopaedic applications.

4:00pm **PS+BI-WeA7 Study of Adhesion Mechanism of Protein-based Hydrogel to Plasma Treated Polymer Surface**, ***O. Zabeida***, Ecole Polytechnique of Montreal, Canada, *M.-P. Faure*, Bioartificial Gel Technologies, Canada, *J.E. Klemberg-Sapieha, L. Martinu*, Ecole Polytechnique of Montreal, Canada

Biodegradable protein-based hydrogels (solid water solutions, SWS^{TM}) are a new class of biomaterials with great potential for use in numerous pharmaceutical and medical applications. Since they may contain up to 96% of water, some SWS are rather fragile and difficult to handle and manipulate. This problem can be solved by applying appropriate polymer backings; the latter one has to be surface treated in order to enhance the hydrogel's adhesion. We found that plasma modification of polymer backings can lead to a 20-fold increase of the adhesion force between the SWS and the polymer surface. In the present work we have applied a multitechnique surface analytical approach, including infrared spectroscopic ellipsometry, XPS, AFM, and TOF-SIMS, to investigate the adhesion mechanism of hydrogels to low pressure plasma-treated polymers (polypropylene, polyethylene terephthalate, and others). The surface chemical structure and morphology are correlated with the adhesion force of the SWS. The results suggest that introduction of amine groups plays a major role in the adhesion improvement, while the surface roughening, polymer chain scission and surface electric charge should also be considered.

4:20pm **PS+BI-WeA8 Permanent Hydrophilic Modification of Porous Membranes Using Low-Temperature Plasmas**, ***D.S. Wavhal***, *E.R. Fisher*, Colorado State University

We have explored the use of low-temperature plasmas to modify porous polymeric membranes with the goal of creating hydrophilic surface throughout the membrane structure. One motivation for this work is to decrease membrane fouling and to eliminate the need for wetting agents in a variety of applications. Porous polyethersulfone (PES) membranes were modified by CO_2 plasma treatment and Ar-plasma treatment followed by grafting of hydrophilic monomers (acrylic acid and acrylamide), in the vapor phase. Plasma treatment and plasma induced grafting rendered a complete hydrophilicity to the entire PES membrane cross section. The hydrophilicity of the membranes treated with only the Ar-plasma is not, however, permanent. In contrast, the PES membranes treated with CO_2 plasma and the grafted membranes are found to be permanently hydrophilic (for a minimum of six months). Chemical changes to the modified PES membranes were determined with FTIR and XPS measurements. Furthermore, water bubble point measurements and electron microscopy results reveal that pore sizes of the modified membranes are slightly affected. The pore sizes of the grafted membranes at higher grafting yield are slightly decreased. Due to incorporation of polar functionalities, the glass transition temperature (T_g) of modified membranes also increases. A moderate change in tensile strength of the modified membranes was observed. Most importantly, the surface of the modified membrane are less susceptible to absorbtion by bovine serum albumin (BSA) proteins and give greater flux recoveries. This suggests that the protein fouling layer is reversible because of hydrophilic nature of the modified membranes.

4:40pm **PS+BI-WeA9 Acrylic Acid Films Deposition by RF PACVD: Relation between Monomer Fragmentation and Surface Properties**, *P. Rossini, G. Ceccone*, European Commission, Joint Research Centre, Italy, *K. Jandt*, University Jena, Italy, ***F. Rossi***, European Commission, Joint Research Centre, Italy

The present study deals with the deposition of acrylic acid thin films by radio frequency plasma assisted chemical vapour deposition. The experiments have been carried out in a cylindrical capacitively coupled plasma reactor at different electrical powers (5-60 Watt), in order to optimise the precursors fragmentation and to tune selectivity and stability of the deposited polymers. In situ diagnostics (Mass Spectrometry and Optical Emission Spectroscopy) have been used in order to control the deposition processes and analyse the fragmentation steps. The films have been characterised with X-Ray Photoemission Spectroscopy (XPS) and Fourier Transformed Infrared Spectroscopy (FTIR). Surface energy of the coatings has been determined by contact angle measurement. The protein adsorption kinetics has been evaluated with the Quartz Crystal Microbalance (QCM-D) with HSA. The results demonstrate a strong link between monomer fragmentation in the plasma and functional groups retention in the films. By increasing the RF power, the COOH concentration in the films (XPS and FTIR) as well as hydrophylicity, hydrogen bondings and acid-base character decrease while the CO concentration in the plasma phase (MS and OES) increases. At the same time, the dispersive and the polar components of the surface free energy increase. These surface properties have a strong influence on the protein attachment kinetics, as determined by QCM measurements.

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