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E-MRS Spring Meeting 2002
June 18 - 21, 2002

SYMPOSIUM R

Microstructured Biomaterial Surfaces

Symposium Organizers:

Rolando Barbucci, University of Siena, Italy

Adam Curtis, University of Glasgow, U.K.

Ann-Christine Albertson, KTH, Stockholm, Sweden

SYMPOSIUM SUPPORT:

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SYMPOSIUM R

E-MRS 2002 SPRING MEETING

SYMPOSIUM R

Tuesday, June 18, 2002
Mardi 18 juin 2002

Morning
Matin

Session I:

- R-I.1** 09:00 **ENDOTHELIAL CELLS BEHAVIOUR ON MICRO AND NANOSTRUCTURED SURFACES**
A. Magnani, Department of Chemical and Biosystem Sciences and Technologies, University of Siena, Via A. Moro 2, 53100 Siena, Italy
The surface control of cellular behaviour such as adhesion, diffusion, migration, proliferation and differentiation plays an important role in the formation of tissues and organs, as well as in the realisation of functional biomaterials. The presence of micro or nanostructures on a surface, allows the control and manipulation of two fundamental external signals: the cell-substrate and cell-cell interactions in such a way to create patterns of cells that are highly oriented and differentiated. Micropatterned surfaces with different chemistry and/or topography were thus synthesised in order to foresee the degree and type of the modulation of processes concerning the biointegration of new micropatterned materials in the host. In particular, glass substrates were modified by micropatterning a polysaccharide: the sulphated hyaluronic acid (HyalS) by the photolithography technique. Four different patterns (10, 25, 50 and 100 μm) were obtained. The microstructured samples were analysed by Infrared Microscopy, Secondary Ions Mass Spectrometry, Scanning Electron Microscopy and Atomic Force Microscopy to understand the chemistry and the topography of the surfaces. The influence of chemistry and topography on the endothelial cells behaviour was analysed in terms of cell adhesion, locomotion and orientation. A polarised movement of the endothelial cells was obtained on the micropatterned materials with the degree of polarisation increasing with decreasing the microdomain size.
- R-I.2** 09:45 **THE AFFECTS OF IRREGULAR NANOTOPOGRAPHY ON MAMMALIAN CELL ADHESION AND BEHAVIOUR**
M.A. Wood, University of Glasgow, UK
Microtopography is one of the four main factors capable of affecting cell adhesion[1]. Recent investigations concerning the affects of regular nanotopographies on cell behaviour have also shown a reduction in cell adhesion[2]. However, are the affects of nanotopography only encountered when features are symmetrical?
Through the utilisation of a natural lithography technique[3] where colloidal gold particles are deposited on a substrate and consequently used as an etch mask, it is possible to create irregularly pillared or beaded nanosurfaces[4].
Both endothelial cells and fibroblasts have exhibited a preference for planar surfaces when confronted with a choice of flat and irregularly pillared nanostructures. Cell morphology is also altered in these cell types when grown on pseudo-random nanopillars. Length and breadth is altered, with cells grown on irregular nanopillars exhibiting fine sensing mechanisms such as microspikes and exaggerated filopodia. Actin appears unable to polymerise on these surfaces, and tubulin is disorganised.
[1] Wilkinson, Curtis Dev. In Nanotechnology 1996, 3, 19-31
[2] Curtis, Casey, Gallagher, Pasqui, Wood, Wilkinson Biophys Chem 2001,94,275-83
[3] Deckman, Dunsmuir Appl. Phys. Lett 1982,41, 377-379
[4] Wilkinson, Riehle, Wood, Gallagher, Curtis Mat Sci Eng C 2002 263-269
- R-I.3** 10:05 **SURFACES PATTERN OF LUMINESCENT Q-DOTS AS CELL RECEPTOR BINDING SITES**
A. Szuecs, University of Heidelberg, Germany
Highly luminescent mono- and bimetal semiconductor quantum nanodots (Q-dots) regularly patterned on substrates, were synthesized and applied as binding sites for single cell receptors in order to study cell adhesion.
CdS and CdS/ZnS Q-dots in the size range of 2-8 nm in diameter were generated in two different colloidal systems. Precursor Cd²⁺ ions containing water pool of "traditional" AOT/isooctane reverse micelles (RM1) and with solid Cd salts loaded core of Poly (styrene-b-2-vinyl-pyridine) (PS-P2VP)/inorganic hybrid reverse micellar system (RM2) were used as nanocompartments for preparation of uniform semiconductor nanoparticles. Particle size was controlled by changing the water content (w) and the Cd salt loading of the core in RM1 and RM2, respectively. Surface patterning was carried out on glass substrate in two different ways. 1, "in situ": by self-organization of the diblock copolymer molecules on the surface. 2, "ex situ": by capping and linking of the in AOT reverse micellar dispersion prepared Q-dots with different thiol molecules to the substrate. QDs on surfaces are used also as binding sites for single cell receptors.
Photo luminescent properties of CdS and CdS/ZnS nanoparticles were investigated by different methods (UV-VIS spectrophotometry, steady-state fluorescence, color luminescence imaging).

SYMPOSIUM R

- R-I.4** 10:25 SOLUTION-ASSISTED TRIBOLOGICAL MODIFICATION OF A BIOMINERAL USING AN ATOMIC FORCE MICROSCOPE
Tom Dickinson, Rizal Hariadi, and Steve Langford, Washington State University, Dept. of Physics, USA
When a surface is subjected to tribological loading, bonds experience time dependent distortions and spatial deformations. In the presence of simultaneous chemical stimulation (e.g., from a solution), this can lead to bond breaking, bond formation, and nuclear rearrangement. We present new studies of combining mechanical and chemical stimuli on a model biomineral: brushite (CaHPO₄·2H₂O), particularly under conditions of solution supersaturation. Thermodynamically, the system tends towards deposition or crystal growth; we show that nucleation and growth on the surface can be controlled on the nanometer size scale using simultaneous mechanical stimulation with an AFM tip. Careful analysis of the noise in the cantilever motion during contact scanning shows that on single crystal surfaces we are very sensitive to the presence of sub-critical cluster formation and re-dissolution, we find that the amplitude of the noise increases by factors of 2-4. We take this as evidence for the presence of these precursors to recrystallization. Furthermore, rich noise spectra are observed on crystal surfaces with low symmetry when one changes the scan direction; we observe modulated signals at frequencies corresponding to calculated times between asperity-lattice row encounters. Again, under supersaturation, the noise levels rise in comparison with pure solvent. Finally, we present structures and surface modifications that can be induced by these mechanical/chemical synergisms.
- 10:45 **BREAK**
- Session II:
- R-II.1** 11:15 MICROPATTERNING OF POLYMER SURFACES FOR CONTROLLED CELL ADHESION PROCESSES
C. Satriano(a), S. Carnazza(b), A. Licciardello(a), S. Guglielmino(b), and G. Marletta(a), (a)Department of Chemistry, University of Catania, V.le A. Doria 6, 95125 Catania, Italy; (b)Department of Microbiology, Genetics and Molecular Sciences, University of Messina, Salita Sperone, 31-Vill.S.Agata, 98166 Messina, Italy
The promotion of cell adhesion and spreading processes on polymeric surfaces activated by ion irradiation is observed for several polymers. In this work the physico-chemical properties of surfaces of poly(ethyleneterephthalate) (PET) and poly(hydroxymethylsiloxane) (PHMS) films were modified by using low energy ion beams with a micrometric lateral resolution. Irradiated patterns with stripes of width ranging between 10 and 100 micron were obtained on the two polymer surfaces by highly focused ion beams. The modification in the surface micro-topography and morphology was measured by Atomic Force Microscopy, while chemical structure and composition were characterized by ToF-SIMS and Small Spot XPS. Finally, the surface energies of unmodified and irradiated area were calculated by wettability measurements.
Fibroblast cells were used to test the cell adhesion and viability on the various patterns. Optical Microscopy allowed to discriminate the lateral resolution effect between PET and PHMS for the preferential cell attachment on stripes of different widths.
Epifluorescence Microscopy revealed different cell morphology and mitotic activity for the different patterned surfaces. Furthermore, preferential cell alignment effects were observed depending on the pattern size.
The observed cell adhesion and spreading behavior is discussed in view of the correlation between the ion-beam induced physico-chemical surface modification and the related biological response.
- R-II.2** 11:35 CONTROLLED PATTERNING OF BIOMOLECULES ON SOLID SURFACES
Etienne Schacht(a), Yves Martel (a), Kristof Callewaert(a), Kris Naessens(b), James Kirkpatrick(c), (a)Department of Organic Chemistry-Polymer Material Research Group, Ghent University, Krijgslaan 281-S4, 9000 Ghent, Belgium and (b)Department of Information Technology, Ghent University and (c)Department of Histopathology, University of Mainz, Germany
Patterning biomolecules on a surface with a geometrical accuracy and high spatial resolution has a great potential in different fields. The cellular attachment and adhesion onto surfaces are important for the study of cell growth and a number of technological applications, such as nerve regeneration, biosensors,.... Laser ablation[1,2] is a powerful flexible technique for rapid patterning of very small features, which can be applied for a number of different polymer materials. Irradiating polymer coatings onto a solid state, e.g. gold, gives us the opportunity to micropattern the photoresist in a controlled way. At the ablated areas the polymer coating is removed and different molecules can be attached at the bare gold surface. Alkanethiols (HS-(CH₂)_n-X), chemisorb on gold and form self-assembled monolayers[3] (SAMs) on the surface. The terminal position, functional groups, of the alkanethiol makes it possible to control the structure of the surface at a molecular level. Reactive endgroups, e.g. carboxylic acid, alcohol, aldehyde, can form interactions (covalently, ionic) with different biomolecules, such as proteins, enzymes, ... We already developed a method to attach enzymes[4], glucose oxidase, in a patterned way onto a gold surface. At present this approach is used to anchor cell interactive groups onto the patterned surface. This can promote the attachment and spreading of cells in a patterned and controlled way. The growth of cells and stability of the deposited cell layer under flow conditions is under investigation. Latest results about the spreading and growth of endothelial cells on these patterned self-assembled monolayers and their resistance to detachment under flow conditions will be presented at the meeting. A method is developed to pattern different biomolecules on a solid surface. By using self-assembled monolayers we can control the growth and spreading of cells on gold surface. This technique can be used for several applications, such as the development of biosensors.
[1] Nishio, S.; Chiba, T.; Matsuzaki, A.; Sato, H. Applied Surface Science, 1996, 106, 132-136
[2] Pola, J.; Urbonova M.; Dinek, V.; Ubrt, J.; Deckers, H. Applied Organometallic Chemistry, 1999, 13 (9), 655-658
[3] Whitesides, G.M. Angew. Chem. Int. Ed. 1998, 37, 550
[4] Gooding, J.J. Trends in analytical chemistry 1999, 18, 525
Acknowledgment : This work was supported by the Flemish Institute for the promotion of Scientific-Technological Research in Industry (IWT), the Fund for Scientific Research - Vlaanderen (FWO) and the Belgian Ministry of Scientific Programming, IUAP-project

SYMPOSIUM R

- R-II.3** 11:55 **CELL BEHAVIOUR ON SURFACES WITH RANDOM AND ORDERED NANOMETRIC FEATURES**
M. Riehl(a), M Dalby(a), H Johnstone(b), J Gallagher(a), A MacIntosh(a), K McGhee(a), S Affrossman(b), (a)Centre for Cell Engineering, IBL & EEE, University of Glasgow, Glasgow G12 8QQ, UK, (b)Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Graham Building, Cathedral Street, Glasgow, G1 1XL, UK
The rationale to fabricate nanometric surfaces for biomedical applications is that cells in the body live within an environment filled with nanometric patterns i.e. collagen I the most abundant extracellular protein arranges into fibrils with 65nm period topography and a repetitive adhesive pattern. Furthermore many biomaterials such as HAPEX™ or nanophase Ti-alloys show intrinsic nanometric surface features. We assumed therefore that nanometric features could serve as a stimulating environment for cells and that such surfaces could be used to guide cells and serve as borders or limits for cellular adhesion.
We have fabricated nanometric pillars and pits using e-beam lithography and reactive ion etching into silicon or fused silica making regular features with 150 and 300nm repeat. For experiments these structures were copied into -polycaprolactone by melt moulding. On the other hand we used a simple method based on phase separating polymers to fabricate random nanotopography to elicit cellular reactions; more specifically we used blends of polystyrene and poly(4-bromostyrene) which undergo phase separation during spin casting onto glass coverslips. Different topographies such as pits, bumps, or ribbons can be fabricated by controlling the solvent concentration and the proportions of the polymers. XPS and SIMS showed that after annealing the structures at 115°C the surface was composed only of polystyrene. For cell experiments we used 13, 35 and 95 nm high islands with flat PS as a control. Rat calvaria bone cells were used as a cell model. The surface structures were analysed using AFM and SEM. The time course of the cellular reactions were analysed using cells fixed at set time points after initiating adhesion.
The artificial surfaces with regular nanometric topography reduced cell adhesion and spreading, whereas the random 13nm and flat PS initially retained more cells than the 35nm and 95nm at 1, 4 and 24h but the number of cells was increased on the later type of structure after 3days in culture. The cytoskeleton of the cells (F-actin MT, vinculin) was best developed on the flat and 13nm random structures, all other features acted disruptive. Cell behaviour was affected by all nanometric features: regular features increased motility compared to flat and random features increased intracellular trafficking (13nm) and lamellipodia formation (95nm) compared to flat control experiments.
In conclusion the use of fabricated nanometric surfaces revealed unexpected and interesting reactions of a variety of cells, from gene expression to shape and behaviour all aspects of a cell seem to be profoundly affected by the fine detail of the surface. The results point to possible uses of such surfaces e.g. to improve medical devices or tissue engineering.
The research was funded by the EU Grant QLK3-CT-2000-01500 (Nanomed). The optical microscope used was acquired with a start-up dowry from IBL (Univ of Glasgow) and further funding from the EPSRC (grant: GR/R15009).
- R-II.4** 12:15 **CARBON NANOCOMPOSITE MATERIALS AS MEDICAL DEPOT**
Galina U. Ostrovidova, Saint-Petersburg Technological University, Russia
Studies on physico-chemical properties of various supports for immobilization of biologically active substances are of principal interest. These studies are currently central because of the need in development of materials for medicine (artificial organs, supports of medicinal substances), problems of protein cleaning as well as modeling of membranes and new biocatalysts. The most important task is to find materials providing high activity of biologically active substances contacting with them, support surface nature and its chemical inhomogeneity, concentration of surface adsorption active centers being not only functional groups, but also nanopores, are the factors, which determine adsorption mechanism and intensity of surface intermolecular interaction [1-2]. In addition to the effect of surface on inactivation of ferments the effect of immobilization conditions (duration, temperature, pH and other technological parameters) is great. High chemical and biological stability of carbon materials, compatibility with blood and tissues of organism enables a successful using of them for immobilization of biologically active substances [3-5].
The goal of the present work is to study the effect of chemical nature of surface and structural features of carbon materials on immobilization of biologically active substances.
As starting materials nanodiamond composite materials were used, which comprise nanodiamond particles bonded by graphite-like matrix [6]. The materials were different by their open porosity and surface area. The surface functional groups have been analyzed and some physical characteristics of the studied carbon materials have been estimated. Using various methods the ferments, namely trypsin and glucose oxidase and antibiotic - canamicine were immobilized. As a result of the estimated ferment- and anti-microbe activity it was determined that the given carbon material may also function as a medicinal depot.
[1] Ostrovidova G.U., Chechot M.I., Denisova O.V., Ivanova E.N. Some physico-chemical properties of modified graphite with immobilized trypsin// *J. of Phys.Chem.*, 1987, V.61, #10, p.2828-2892
[2] Denisova O.V. and Ostrovidova G.U. Dependence of bonded trypsin activity on structure and chemical composition of carbon supports// *J. of Phys.Chem.*, 1992, V.66, #3, p.744- 752
[3] Bokros J.C., Le Grange L.D., Schoen P.U. The Graphite Structure Control for use of Biotechnic// *Chemistry Physics of Carbon*, 1973, V.9, p.104-171
[4] Razumas V.I., Yasaitis Yu.Yu., Kulis Yu.Yu. Amperometrical ferment electrodes based on carbon materials. *Vilnyus*, 1984, 45 p.- Dep. at VINITI, 23.03.84, #1609-84
[5] Ostrovidova G.U. Scientific basics of designing of artificial organs// In book: "Directed synthesis of solid substances" L. :LGU, 1987, #2, p.142-150
[6] Gordeev S.K. Nanoporous and Nanofragmental Carbon Composite Materials/ in G. Benedek et al (eds.), *Nanostructured Carbon for Advanced Applications*, Kluwer Academic Publishers, 2001, p 71-88.
- 12:35 **LUNCH**

SYMPOSIUM R

Tuesday, June 18, 2002
Mardi 18 juin 2002

Afternoon
Après-midi

Session III

- R-III.1** 14:00 MEASUREMENT OF CELLULAR FORCES AT FOCAL ADHESION USING ELASTIC MICROPATTERNED SUBSTRATES
Ulrich Schwarz, Max-Planck Institute of Colloids and Interfaces, Germany
Mechanical force is known to play an important role in the regulation of cellular behavior, including adhesion, motility, differentiation and proliferation. For stationary, mechanically active cells like fibroblasts, adhesion to flat substrates occurs mainly at sites of focal adhesions, which are micron-sized protein aggregates at the plasma membrane which on the cytoplasmic side connect to the actin cytoskeleton. In recent years, evidence has been growing that focal adhesions act as mechanosensors which convert mechanical force into biochemical signalling. We have investigated the relationship between force and aggregation at focal adhesions by a new method which combines elastic micro-patterned substrates (to record substrate deformation), fluorescence labelling of focal adhesion proteins (to monitor aggregation) and numerical solution of the inverse problem of linear elasticity theory (to calculate forces at focal adhesions). We have found that force correlates linearly with lateral size of aggregation, with a stress constant of 5.5 nN/square micron. This finding indicates that mechanosensing involves regulation of aggregation.
- R-III.2** 14:45 CELL ATTACHMENT AND SPREADING ON METAL IMPLANT MATERIALS
Ellen S. Gawalt(a), Brett M. Silverman(a), Kim Midwood(b), Jean E. Schwarzbauer(b), Michael A. Avaltroni(a), Michael P. Danahy(a), Jeffrey Schwartz(a), (a)Department of Chemistry, Princeton University, Princeton NJ 08544-1009, USA, (b)Department of Molecular Biology, Princeton University, Princeton NJ 08544-1014, USA
Titanium and its alloys enjoy widespread use as surgical implants, many of which contact bone. Paradoxically, the high corrosion resistance of Ti and its alloys can be problematic in a clinical context: The bone-to-implant interaction is weakened by the absence of strong chemical bonding at the interface between the implant surface and the mineral and organic components of the bone. Thus, osteointegration is reduced, implants fail under shear stress, and revision surgery is required. One approach to preparing an osteoconductive interface involves bonding the fibronectin cell attachment peptide RGD to the implant surface through an organic tether. Silanization of the native oxide coating of Ti or Ti-6Al-4V has been the most widely used means to attach the RGD-binding tether to these critical materials, but only low surface coverage has been effected. We have reported two novel means to interface organics with Ti or Ti-6Al-4V; neither is limited by the OH group coverage of the native oxide surfaces; both enable comprehensive substrate surface coverage. We will describe strong bonding and coverage of RGD which is far greater than can be accomplished using conventional methods and which can be readily effected on both Ti and Ti-6Al-4V. We will show our surfaces promote considerable surface attachment and spreading of fibroblasts on Ti and Ti-6Al-4V. Thus, we have now achieved an important milestone in making actual implant surfaces osteoconductive.
- R-III.3** 15:05 BONE TISSUE ANALYSIS USING INFRARED SPECTROSCOPIC
J.-C. Cigal, G.M.W. Kroesen, R. Huiskes, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, The Netherlands
Osteoporosis is defined as a systemic disease causing an absolute decrease in the amount of bone and microstructural changes. This concerns 30% of the women of 50 years and older. Although many factors have been shown to be correlated to osteoporosis, the mechanism of the development of the disease remains unclear. Among the precursor signs of osteoporosis is the development of micro-cracks at the surface; their size is typically few microns. Furthermore a change in the chemical composition of the bone tissue around these cracks is suspected. Our group is developing a Fourier transform spectroscopic ellipsometer in the mid-infrared range (2.5 to 10 microns). Ellipsometry is a highly sensitive and non intrusive method for surface analysis. Our ellipsometer is very suitable for the analysis of the surface of the bone tissue. Moreover a spectroscopic study will provide information on the chemical composition of the bone tissue. In order to investigate the possibility of treating it, the bone is exposed to a low-pressure gas discharge (inductively coupled plasma). The tissue is analyzed before and after treatment. We have already shown some changes in the structure on the bone due to the plasma exposure, ellipsometry can give more information on this phenomenon. An in-situ investigation is also planned. Preliminary results will be presented.
- R-III.4** 15:25 SURFACES ENERGETIC ENGINEERING OF PECVD POLYMERIC FILMS FOR BIOMEDICAL APPLICATIONS: BACTERIAL AND CELL ADHESION RESPONSE
P. Rossini, EC-JRC-IHCP, Ispra, Italy, G. Ceccone, EC-JRC-IHCP, Ispra, Italy, F. Rossi, EC-JRC-IHCP, Ispra, Italy
Organic films (Polyethylene-like, Polyacrylic acid-like and Polyethyleneglycole-like) with different surface free energy have been deposited by plasma enhanced chemical vapour deposition (PECVD) in order to study their bio-reactivity (proteins adsorption, bacterial adhesion, cell seeding) when in contact with biological fluids (blood, saliva).
Plasma and surface diagnostics have been used to optimise and control the deposition processes. For the study of the plasma phase, Mass Spectrometry, Optical Emission Spectroscopy and Fourier Transformed Infrared Spectroscopy (FTIR) have been used, whilst the films have been characterised by using, X-Ray Photoemission Spectroscopy (XPS) and FTIR.
The coatings superficial properties (surface energy) have been studied by means of contact angle measurements, while their bioreactivity when in contact with biological fluids so with proteins, cells, bacteria has been investigated by a Quartz Crystal Microbalance by monitoring their absorption kinetics. Moreover, optical and electron microscopy have been used to observe the influence of biological interactions on films morphology. The relation between film deposition parameters and biological response will be discussed.

SYMPOSIUM R

- R-III.5** 15:45 REGULATION AND CONTROL OF STRUCTURE AND RELIEF OF PARTICLES SURFACE OF HYDROXYAPATITE NANOPOWDERS FOR USE IN DIFFERENT MEDICAL APPLICATIONS
V. Dubok, A. Shevchenko, E. Shevchenko, A. Shikaruk, Institute for Problems of Material Science Ukrainian Academy of Science, Kiev, Ukraine; A. Karlash, K. Yakovkin, T. Veblaya, A. Gorchinskiy, G. Zykov, E. Buzaneva, National T. Shevchenko University, Kiev, Ukraine
To develop new generation of hydroxyapatite (HAP) powder implants for bone tissue restoration as well as materials for drug delivery systems and prolongation of drug action, it is important to regulate the total value of surface area of the nanopowders and both to achieve selective or nonselective activation of these powders in different chemical and biochemical reactions. With the purpose to find ways to resolve these problems the methods were investigated to regulate the structure and relief of particles' surface of HAP powders. Regulation of surface area of the powders were attained by choosing of parameters and compositions for of hydrothermal - chemical treatment of products of HAP chemical deposition. To obtain selective pattern of a particle surface relief, the idea have been utilized to create surface relief with shape which is complementary to some molecules to provide "key and lock" principle for their recognition.
The reflectometry, IR spectrometry, XPS, Scanning Force Microscopy, Laser Mass Spectroscopy were used.
- 16:05 **BREAK**
- Session IV
- R-IV.1** 16:35 OSTEOBLAST BEHAVIOR ON NANOSTRUCTURED TITANIUM ALLOYS
Achille Cittadini(a), Alessandro Sgambato(a), Ana Dardeli(a), Alessandro Facchini(b), Paolo Dalla Pria(b), Andrea Colombo(b), (a)Giovanni XXIII - Cancer Research Center, Catholic University of Sacred Heart, Roma, Italy, (b)Lima Lto spa, San Daniele, Udine, Italy
In this study, new biocompatible nanostructured materials made of titanium alloys have been developed, manufactured and studied in term of their biocompatibility. The major feature of such materials is to comprise structures with zero dimensionality (groups of atoms "clusters"), monodimensioned multilayers and nanofasic solid or three-dimensional nanocrystals. Moreover, they theoretically have no limitations of chemical composition.
Various samples with different composition have been realized, varying the parameters of the manufacturing process and amongst them some have been chosen for further studies since they appeared more suitable for the specific demanded applications. On these selected materials metallographic and roughness analyses have been performed. Moreover, with the cooperation of a specialized University Institute, cytotoxicity tests (both for direct and indirect contact) have been performed and the ability of osteogenic cells to attach, grow and differentiate on this materials has been analyzed in vitro. The results obtained show that even small granulometric variations of few nanometers influence the biocompatibility of these materials in term of cytotoxicity and in term of osteogenic cells attachment and grow. These data are important since they likely reflect the behavior of osteoblast cells and relate to bone remodeling in vivo.
- R-IV.2** 16:55 ITEMS ON THE BASE OF NANOCOMPOSITE MATERIALS FOR MEDICAL APPLICATION
Galina U. Ostrovidova, Saint-Petersburg Technological University, Russia
At present in medical practice there is the lack of materials for implants. Metallic, ceramic and polymeric materials not completely correspond to the demands of the medicine.
The problem of the creation of implants with beforehand planing complexes of properties may be solved on basis of use of composite materials.
It is well-known that carbon materials represent the most interest for use in medicine. In Saint-Petersburg State Institute of Technolojy (Technical University) the novel technology of the production of carbon containing composite materials of different appearance, such as carbon-polymer and carbon-metal is worked out. Non-toxic, non- cancerogenic, thromboresistance and chemical inertia of the materials as a medical goods are the advantages of this technology in comparison with well-known analogies.
The technology of creation of the material was verified in conditions of industrial production. The level of mechanical, biological, physico-chemical properties of the composite materials was tested and the possibility of their directed alteration, taking into account obtaintion of the items allowing to creation on their base of blood-vessels, tracheas and bronchus implants, as well as temporary medical products, different stomatological and inserts for the fingers of the upper limbs was established.
- R-IV.3** 17:15 METHODS FOR THE PHYSICAL AND CHEMICAL CHARACTERISATION OF SURFACES OF TITANIUM IMPLANTS
A. Kirbs, R. Lange, B. Nebe, F. L., then, J. Rychly, U. Beck, University of Rostock, 18051 Rostock, Germany
At present one is still searching for physical and chemical methods which are suitable for the characterisation of the biocompatibility of implant surfaces. In this paper we analysed various micro structured titanium surfaces (the roughness average of the various structures differs by four orders of magnitude) with a number of electrochemical and physical methods such as electrochemical impedance spectroscopy, chronoamperometry, voltammetry, surface profiling and scanning electron microscopy. The aim of the work is a comparison of the specific methods regarding to their significance and correlation of the results. Beside the extraction of physical and electrochemical parameters which are typical for the above mentioned methods the possibility of the determination of the fractal dimension of the surface structures is also examined. Cell biological examinations concerning the mechanisms of cell adhesion were used to verify the ability of the parameters measured by the referred methods to describe the biocompatibility of the analysed surfaces. Osteoblastic cells were cultured on the differently structured titanium surfaces and we determined spreading and adhesion of the cells. These parameters revealed significant differences in dependence on the surface topography.

SYMPOSIUM R

- R-IV.4** 17:35 **MICROPATTERNED POLYSACCHARIDES SURFACES VIA LASER ABLATION FOR CELL GUIDANCE**
Rolando Barbucci, Stefania Lamponi and Daniela Pasqui, Department of Chemical and Biosystem Science and Technology, University of Siena, Via A. Moro 2, 53100 Siena, Italy
Micropatterned materials were obtained by a controlled laser ablation of a photoimmobilised homogeneous layer of the polysaccharides. Hyaluronic acid and its sulphated derivative, adequately functionalised with a photoreactive group, were photoimmobilised on aminosilanised glass substrate and subjected to laser ablation with wavelengths in U.V. regions. The material removal is obtained mainly through the breaking of chemical binds followed by collision waves which induce material extraction. Four different patterns having stripes of 100, 50, 25, 10µm were realised. Chemical characterisation (ATR/FT-IR, TOF-SIMS) confirmed that the laser ablation was successful and the absence of the polysaccharides on aminosilanised glass. Morphology analysis (A.F.M, S.E.M.) revealed the exact dimensions of the stripes. The analysis of cell behaviour was performed using human endothelial cells in terms of adhesion, proliferation and movement.
- R-IV.5** 17:55 **THE DETERMINATION OF THE MORPHOLOGY OF MELANOCYTES BY LASER GENERATED PERIODIC SURFACE STRUCTURES**
R. Kemkemer and J. Spatz, Biophysical Chemistry, Institute of Physical Chemistry, University of Heidelberg, INF 253, 69122 Heidelberg, Germany, M. Csete, Department of Optics and Quantum Electronics, University of Szeged, Dóm tér 9, 6720 Szeged, Hungary, S. Schrank, Department of Biophysics, and D. Kaufmann, Department of Human Genetics, University of Ulm, Albert Einstein Allee 11, 89069 Ulm, Germany
We generated self-organized, grating-like structures on the surface of polyethylene-terephthalate by ArF excimer laser ablation. Tilting the polymer films with angles larger than 60° we got parallel grooves. The surface changes caused by the laser illumination were qualified by AFM. The period of the structures was approximately 1 micrometer, which is commensurable with the characteristic size of melanocytes. We examined the cell shape of normal epidermal melanocytes (M-C), melanocytes from the skin of one neurofibromatosis 1 patient (M-NFS) and from a café au lait macule from another patient (M-NFC) in vitro. The number of dendrites per cell, the length of dendrites, and the orientation of several hundred cells were evaluated by an image-processing program. It was proven that the cell morphology and orientation is determined by the topography of the structured polymer substrate. All cells are aligned parallel to the grooves and show the typical bipolar shape. In contrast, the NF cells on the untreated part of the substrate have many branches and are randomly oriented

SYMPOSIUM R

Wednesday, June 19, 2002
Mercredi 18 juin 2002

Afternoon
Après-midi

14:00-15:00 POSTER SESSION

- R/P.01** INVESTIGATION ON THE INTERACTION PLASMA-BONE TISSUE
C.Y.M. Maurice, J.H.R. Feijen, E. Wagenaars, E. Stoffels, G.M.W. Kroesen, Eindhoven University of Technology, The Netherlands
These last decades, successful plasma modifications of the surface of biomaterials have already been obtained, such as for example: coatings with polymer layers, enhanced wettability or adhesion, improved biocompatibility or surface functionalization. However, the actual consequences of plasma interactions with organic matter have not yet been resolved. In this work we investigate the impact of positive ions formed in a low-pressure plasma on bone tissue surface. The sample is subjected to controlled ion bombardment in an Inductively Coupled Plasma source and the post treatment responses are investigated using Scanning Electron Microscopy. To monitor the ion energy, an energy resolved mass spectrometer is placed at the plane of the sample on the bombarded electrode and records the Ion Energy Distribution Functions. A Langmuir probe gives densities and potential measurements in the bulk plasma and the DSLIF (Doppler Shifted Laser Induced Fluorescence) technique gives access to IVDFs (Ion Velocity Distribution Functions) of the plasma ions. Plasma etched products are recorded with the mass spectrometer while processing the bone samples. Several noble gases were used to investigate grades of low-energy bombardment and input parameters (power, pressure, bias) were varied to modify densities and energies. Understanding plasma-bone tissue interactions under vacuum conditions could help for designing a non thermal atmospheric plasma source for organic treatment.
- R/P.02** LOCAL BONDING GEOMETRY OF GLYCINE ON Cu(100) AND Cu(110)
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Using scanned-energy mode photoelectron diffraction (PhD) from the O 1s and N 1s photoemission we have studied the local structure of glycine on Cu(100). In common with our earlier study of this species on Cu(110) [1] we find a good description of the data with the N atom near atop a surface Cu atom and the O atoms in equivalent off-atop sites, consistent with bidentate bonding of the carboxyl group and additional bonding through the amine. Steric considerations, however, highlight a significantly higher packing density on Cu(100) than on Cu(110) likely to involve intermolecular interactions which may differ in the two cases, encouraging us to explore other possibilities. Removing the constraint that the two O atoms are symmetrically equivalent leads to no improvement of the fit to the PhD data on Cu(100), but reanalysis of the data on Cu(110) shows that an even better fit can be obtained by allowing this distortion, although the essentially bidentate character of the bonding is retained, consistent with other published work using vibrational and X-ray absorption spectroscopy. No such characterisation data are available for the Cu(100)/glycine system, so we have also considered the possibility of monodentate carboxyl bonding. Our results show that this structure also gives a good description of the PhD data with the bonding O in the same local off-atop geometry on the surface but with reduced molecular vibrational amplitudes.
- R/P.03** OPTIMIZING THE STABILITY OF COLLAGEN COATING OF METALL IMPLANTS BY BIOCHEMICAL MODIFICATION
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Aim: The effect of different grades of crosslinking in collagen on the adhesion, proliferation of human mesenchymal stem cells (hMSC) and the enzymatic stability of these layers were investigated.
Methods: Fibrous collagen I in very thin layers was incubated in carbodiimide (1/8%, 1%, 2%) to modify the grade of crosslinking. After sterilization, an enzymatic degradation with collagenase was performed (1min up to 24h of enzymatic use) and the remaining amount of collagen was defined by densitometry. The biocompatibility was tested by using hMSC and defining their proliferation and adhesion activity. Noncrosslinked collagen served as control.
Results: Noncrosslinked collagen was not detectable after 1 min collagenase. Using 1/8%-1% carbodiimide, a proportional increase in enzymatic stability was found. Using 2% carbodiimide, 100% of collagen were detected after 60 min collagenase. In separate investigations (5d of collagenase) 75% of collagen could be detected. Cellular adhesion and proliferation were not affected negatively by crosslinking.
Conclusion: By crosslinking of collagen, a significant increase in enzymatic stability can be achieved. By varying the concentration of the crosslinking reagent the stability can be changed to a desired optimum without loss of cellular biocompatibility.
- R/P.04** HYDROXYAPATITE COATING OF POLYELECTROLITE HYDROGELS BY MEANS OF THE BIOMIMETIC METHOD
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The bioceramics that are able to bind to living bone are called bioactive ceramics[1]. The reaction with blood plasma causes the formation of an hydroxyapatite surface layer where the osteoblasts can adhere and proliferate. It was recently discovered [2] that the formation of the same kind of surface hydroxyapatite layer on both metallic than polymeric supports can be obtained by means of the so called biomimetic method, consisting of two steps. Hydroxyapatite nucleation occurs in the first step when the support is soaked into a simulated body fluid (SBF) resembling the composition of the blood plasma, in contact with glasses of appropriate composition. The successive soaking of the support into a solution having all the ionic concentrations of the SBF increased by 50%, called 1.5SBF, allows to obtain a dense and uniform layer of bone-like apatite. The ability of the method was demonstrated [2] for several polymeric and metallic supports.
In this paper the results are reported of the application of the method to cationic and anionic polyelectrolytes. These were obtained by means of bulk radical copolymerization of 2-hydroxyethyl methacrylate (HEMA) with 2-metacryloxyethyltrimethylammonium chloride as cationic monomer or 2-acrilammido-2-metilpropan sulfonic acid as anionic monomer.
[1] L. Hench, J. Amer. Ceram. Soc. 74 (1991) 1487.
[2] T. Kokubo et al., in Bioceramics; Vol.4, (Butterworth-Heinemann, Guilford, London, 1991) p.113.

SYMPOSIUM R

- R/P.05** INTERFACE CHARACTERISTICS CHANGED BY HEAT TREATMENT OF Ti MATERIALS WITH HYDROXYAPATITE
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The microstructure and chemical composition in the interfaces after heating of pure Ti(Grade 2) and Ti6Al4V buried in HA powder and after sintering of hydroxyapatite(HA) dip coated-Ti based materials, were investigated by SEM and scanning Auger electron spectroscopy. The cylindrically shaped specimens were heated at 800, 850 and 900°C for 1 hour in a protective argon atmosphere. The HA-Ti interfaces were examined after removing of the adhered HA powders and HA coating layers with a jet spray of pure water. The heating led to the formation of porous surfaces, the pore spaces increased with the grain growth caused by the elevated temperature. The phosphorus diffused beyond the titanium oxide layers which act as an excellent "gettering effect" for phosphorus. P peak was observed at 120 eV, indicating the formation of a Ti phosphide. The phosphorus concentration diffused from the HA coating layer after heating at 800°C; was increased up to 7 at.%. The P concentration obtained from this sintering process was 3 times higher than that formed by heat treatment of Ti materials buried in HA powder. In the interfaces heated at 900°C, high oxygen concentrations were present, and little P and Ca concentration were observed. This low-cost and simple surface modification technique of titanium materials may provide a biomaterial for hard tissue replacements.
- R/P.06** ON THE DESCRIPTION OF THE FRACTAL NATURE OF MICRO STRUCTURED SURFACES OF TITANIUM-IMPLANTS
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The determination of the fractional dimension is an interesting way for the characterisation of rough surfaces. Microstructured titanium surfaces produced by different technologies (polished, glass and corundum blasted, vacuum plasma sprayed) were investigated by a number of physical and electrochemical methods i.e. surface profiling, scanning electron microscopy, chronoamperometry, voltammetry and electrochemical impedance spectroscopy. The data sets obtained by these very different methods were used for estimation of the fractal dimension of all structures. The aim of this work is to examine the suitability of this additional parameter for a better characterisation of structured surfaces and to compare the various applied methods regarding their feasibility. Accompanying cell biological examinations were used to verify whether the fractal dimension can be a relevant parameter for the description of the biocompatibility. We analysed the morphology of the cells using scanning electron microscopy and demonstrated differences in the expression and distribution of integrins and in the expression of fibronectin in dependence on the roughness.
- R/P.07** BIOMIMETIC GROWTH OF HYDROXYAPATITE FILMS AT SUBSTRATES MODIFIED BY STEARIC ACID MONOLAYERS
Natali I. Korovnikova, Yuri N. Savin and Alexander V. Tolmachev, Svetlana V. Vitushkina, Alexandra S. Kryzhanovska
The bioceramic films on the basis of Hydroxyapatite (HAP) have a wide application as a advanced orthopedic and dental implants because HAP elicit a favorable biological response and forms a bond with a surrounding tissues. In this communication we report about a forming of HAP films by the biomimetic processes. The HAP films with a thickness 0,5 micrometers were grown from aqueous solutions of Calcium chloride, Sodium phosphate (dibasic) and Sodium hydroxide. In accordance with TEM- and IR-measurements the films had the amorphous structure of Calcium phosphate. After a treatment in oxygen atmosphere at 1120 K during two hours the films structure changed to polycrystal one of HAP. The effect of the initial concentrations of precursors, pH and temperature aqueous solutions as well a film treatment temperature and duration in oxygen atmosphere on the elemental composition and film structure are discussed.
- R/P.08** EFFECT OF TUNGSTEN 0-8% WEIGHT ON THE MICROSTRUCTURE OF THE Co-Cr ALLOYS USED IN THE ODONTOLOGICAL APPLICATIONS
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Alloying elements, such as W, Mo, Mn, are of a large importance in the preoxidation of dental alloys and consequently on the quality of ceramic-metal bond. This study deals on the effect of the addition of tungsten on the microstructural state of dental alloys base on Co-Cr, before the ceramisation process. These materials were prepared by unidirectional solidification. Their characterization, using transmission electronic microscopy and X rays diffraction, shows that the addition of tungsten up to 8% weight involves microstructural changes and the appearance of new phases which are linked to the added content of tungsten element.
- R/P.09** A ROBUST MULTI-PLATFORM MICROARRAY SURFACE ATTACHMENT CHEMISTRY
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Available attachment chemistries for immobilisation of nucleic acid probes on microarray substrates do not allow removal of hybridised target DNA without simultaneous removal of a proportion of the immobilised probes. This limitation increases both cost and variability of microarray-based gene expression analysis. We have developed a novel and robust attachment chemistry that can be used on a range of substrates which enables microarrays to be reused. This was accomplished by simultaneous optimisation of substrate cleaning and derivatisation, selection of an optimal linking molecule to minimise steric hindrance during hybridisation, selection of an optimal printing buffer for spot morphology and consistency, and covalent attachment of the DNA molecule. This attachment chemistry is versatile enough to be applicable to a range of substrates, and enables target DNA to be removed and microarrays to be reused several times without a significant loss of signal.
- R/P.10** ANALYSIS OF GE NANOSTRUCTURES GROWN ON Si(111)
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We study by Scanning Tunneling Microscopy (STM) the formation and evolution of 3D islands obtained by Physical Vapor Deposition of Ge on Si(111) in the temperature range 450-550 C. The early stages of growth are followed by an STM movie showing the formation of triangular, 2D islands that progressively increase in density and dimension, up to completion of the Wetting Layer (WL). This was done by scanning the surface while evaporating Ge at low flux. On this WL 3D coherent island nucleation begins at a Ge coverage between 3-5 MonoLayers, depending on conditions. At T = 500 C the islands (average lateral dimensions 150-200 nm) appear as truncated tetrahedra. They first evolve by introducing new crystallographic faces and by becoming more rounded in shape, and subsequently by including dislocations at their border, partially relieving the misfit strain. Finally an erosion of the islands' top and of the substrate around the islands occurs, probably connected to a substantial Ge-Si intermixing. This is consistent with previous X-Ray Absorption Fine Structure measurements. We analyze the self-organization of the islands, observing that two growth regimes arise: initially islands form and evolve only on steps, up to complete ripening; subsequently the same happens on flat areas of the sample. For Ge deposited at 450 C, the average distance between islands and steps is nearly constant, forming a single row of equally spaced islands, followed by rows in between.
- R/P.11** TESTING SOL-GEL CaTiO₃ COATINGS FOR BIOCOMPATIBLE APPLICATIONS

SYMPOSIUM R

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In the present work, CaTiO₃ films with thicknesses of 1 μm have been prepared by sol-gel spin coating process. The films have been characterised by XRD, FTIR and SEM to study the chemical, structural and morphological changes produced by the annealing treatments. Polycrystalline coatings with a perovskite structure have been obtained at temperatures of 500°C. These films grown on mirror-like surfaces show good adherence and develop a rough microstructured surface. Their biomimetic properties were assayed by evaluating the growth of apatite in simulated body fluids. We conclude that the coatings are of potential interest in order to enhance the surface properties of Ti based prosthetic alloys.

R/P.12

INVESTIGATION OF CELL REACTIONS TO MICROSTRUCTURED IMPLANT SURFACES

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In the last years the possibilities for generation of structures on implant surfaces with well defined geometry have been greatly enhanced by the adaptation of methods initially developed in semiconductor technology. Such surfaces with defined microstructures may be useful for enhancement of the stable anchorage of transcutaneous implants in connective tissue or prevention of epithelial downgrowth and subsequent exfoliation. Aim of the project is the improvement of dental and other transcutaneous implants by investigation of basic mechanisms of cell orientation and behaviour on microstructured surfaces.

Microstructured titanium experimental surfaces with alternating grooves and ridges in the range between 2 and 50 μm were manufactured by photolithographic processing of silicon substrates, replication in epoxy resin and subsequent coating with titanium. This was done in order to describe the influence of the geometric and functional modifications of the artificial surface and to visualize focal adhesion points at the interface between this surface and the cell membrane by means of fluorescence and confocal laser scanning and electron microscopy using antibodies against adhesion and cytoskeletal molecules. Results dealing with cellular reactions to the surface modifications alone and in combination with biological adhesion factors are presented.

R/P.13

REGULATION AND CONTROL OF STRUCTURE AND RELIEF OF PARTICLES' SURFACE OF HYDROXYAPATITE NANOPOWDERS FOR USE IN DIFFERENT MEDICAL APPLICATIONS

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To develop new generation of hydroxyapatite (HAP) powder implants for bone tissue restoration as well as materials for drug delivery systems and prolongation of drug action, it is important to regulate the total value of surface area of the nanopowders and both to achieve selective or nonselective activation of these powders in different chemical and biochemical reactions. With the purpose to find ways to resolve these problems the methods were investigated to regulate the structure and relief of particles' surface of HAP powders. Regulation of surface area of the powders were attained by choosing of parameters and compositions for of hydrothermal - chemical treatment of products of HAP chemical deposition. To obtain selective pattern of a particle surface relief, the idea have been utilized to create surface relief with shape which is complementary to some molecules to provide "key and lock" principle for their recognition.

The reflectometry, IR spectrometry, XPS, Scanning Force Microscopy, Laser Mass Spectroscopy were used.

R/P.14

NEW CATIONIC POLYELECTROLYTE HYDROGELS FOR BIOMEDICAL APPLICATIONS

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Poly (2-hydroxyethyl methacrylate) p(HEMA) is a widespread used biomaterial that does not allow cell adhesion and growth on its surface limiting the use in the biomedical application in which the cell cohesion is detrimental [1,2]. Actually, we have modified a pHEMA hydrogel by radical copolymerization of neutral acrylic monomer 2-hydroxyethyl methacrylate (HEMA) with cationic monomer 2-methacryloyloxyethyltrimethyl ammonium chloride (METAC) at the aim to keeping such properties as high hydrophilicity and good cell adhesion and growth.

Polymer surfaces were examined by X-ray photoelectron spectroscopy (XPS). Using this technique, we detected an increasing of METAC mole fraction respect to feed composition.

Swelling experiments in pure water of cationic copolymers showed an absorption more marched respect to p(HEMA). Besides, in NaCl solution cationic materials showed a drastically decrease of water absorption.

In buffer solution at the increase of pH, cationic polymers showed a decreased volume.

Finally, citocompatibility studies have showed the non toxicity of synthesized materials with a good adhesion and proliferation for all tested cells.

These results make synthesized copolymers, potentially, employable as biocompatible materials in the area of controlled release devices and dental adhesives.

[1] Wilson A.D., Clinical Materials, Vol. 7, p. 275 (1991).

[2] Chirila T.V., TRIP Vol.2 No. 9, pp. 296-300 (1994).

SYMPOSIUM R

R/P.15

MACROPOROUS POLYELECTROLYTE HYDROGELS FOR TISSUE ENGINEERING

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Poly (2-hydroxyethyl methacrylate) hydrogel is a promising material for orthopaedic application as a stabilizing interface between bone and implant [1].

Recently it has been found that bioactive glasses can help to form a hydroxyapatite layer, by biomimetic method, on the surface of many biomaterials, both organic and inorganic [2].

New macroporous cationic and anionic polyelectrolytes were obtained by bulk radical copolymerization of neutral monomer 2-hydroxyethyl methacrylate (HEMA) with 2-methacryloyloxyethyltrimethylammonium chloride as cationic monomer and 2-acrylamido-2-methylpropan sulfonic acid as anionic monomer in the presence of a filler that was removed at the end of polymerization process.

In our research, we studied the hydroxyapatite formation and the adhesion and proliferation of murine osteoblasts on synthesized materials.

In particular, we had evaluated the effect that polyelectrolytes charges play on the formation of hydroxyapatite crystals on polyelectrolyte surfaces and in the inner structure of macroporous networks.

Finally, we showed that osteoblast adhesion and proliferation take place more rapidly on synthesized materials.

These topics represent important goals for the use of these new materials as scaffold for tissue engineering of bone.

[1] P.A. Netti et al., Biomaterials 14 (1993) 1098.

[2] L. Hench, J. Amer. Ceram. Soc. 74 (1991) 1487.

R/P.16

SURFACE AND INTERFACE ANALYSIS OF HYDROXYAPATITE/TiO₂ BIOCOMPATIBLE STRUCTURES

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The properties of hydroxyapatite (HAP) coatings deposited onto sol-gel derived TiO₂ coatings are evaluated for applications involving the enhancement of biocompatible prostheses. HAP coatings were initially characterised by XRD to confirm the absence of calcium phosphate phases with lower stability at physiological pH. The surfaces of these HAP/TiO₂ structures were subsequently analysed in a two fold manner. A morphologic evaluation of the microstructured surface was made by using surface force microscopy before and after a chemical etching process in acetic acid. Furthermore, the HAP/TiO₂ interface composition could be analysed in depth by using Auger electron spectroscopy. In the case of structures grown onto TiAlV alloys, the composition profiles confirm the presence of apatite nuclei onto the surface, even after etching in acidic conditions. The properties derived from these analyses support the appropriateness of these structures for the development of orthopaedic devices.

R/P.17

SURFACE PATTERN OF LUMINESCENT Q-DOTS AS CELL RECEPTOR BINDING-SITES

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Highly luminescent mono- and bimetal semiconductor quantum nanodots (Q-dots) regularly patterned on substrates, were synthesized and applied as binding sites for single cell receptors in order to study cell adhesion. CdS and CdS/ZnS Q-dots in the size range of 2-8 nm in diameter were generated

in two different colloidal systems. Water pool containing precursor Cd²⁺ ions of "traditional" AOT/isooctane reverse micelles (RM1) and with solid Cd salts loaded core of Poly (styrene-b-2-vinyl-pyridine) (PS-P2VP)/inorganic hybrid reverse micellar system (RM2) were used as nanocompartments for preparation of uniform semiconductor nanoparticles. Particles size was controlled by changing the water content (w) and the Cd

salt loading of the core in RM1 and RM2, respectively. Surface patterning was carried out on two different ways. 1, "in situ": by self-organization of the diblock copolymer molecules are on glass substrate. 2, "ex situ": by capping and linking of the in AOT reverse micellar dispersion prepared

Q-dots with different thiol molecules to the glass substrate. QDs on surfaces

are used also as binding sites for single cell receptors. Photoluminescence properties of CdS and CdS/ZnS nanoparticles were investigated by different methods.

R/P.18

DEPOSITION OF Ti BASED COATINGS WITH DIFFERENT SURFACE STRUCTURE AND CHEMISTRY FOR MEDICAL DEVICES

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Titanium alloys with small amounts of Aluminium and Vanadium are by now the favourite base materials for orthopaedic implants for their good biocompatibility, but stainless steel is wider in use because of its lower material costs and easier handling. By Metal Plasma Immersion Ion Implantation and Deposition (MPIID) coatings of Ti, TiN, Ti_xAl_{1-x}, and

Ti_xAl_{1-x}N layers were deposited on mirror polished stainless steel with a different process parameters, i.e. the bias voltage was changed systematically.

Coatings produced with a bias voltage of -2000 V had a roughness up to 70 nm, whereas those with zero bias voltage were much smoother (6 nm). In coatings with higher voltage the ratio Ti : Al shifted to Ti. Rat one marrow cells were seeded out on these surfaces in a serum free medium. After 5h cell spreading and organisation of the cytoskeleton was best on the smoother samples with best spreading on Ti and Ti_xAl_{1-x}N with 0 bias voltage. There was no significant necrosis as measured by lactate dehydrogenase release within 5 h, but up to 25% pyknotic and fragmented cell nuclei as signs of apoptosis. This behaviour was different for the different samples, but there was no correlation with the surface roughness or chemistry. The effect of the medium, direct induction of apoptosis by the coating or apoptotic cell death due to inhibited adhesion by roughness or surface chemistry will be discussed.

SYMPOSIUM R

- R/P.19** QUANTITATIVE ANALYSIS BY EQCN OF ALKANETHIOL ADSORBED ON MODIFIED TITANIUM SURFACES
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It is generally accepted that the specific protein ligands adsorbed on the biomaterial surfaces enhance the interactions between cell receptors and the surfaces. Little attempt was made to investigate the relation between protein adsorption behavior and the surface chemistry of metallic implant materials. In this study we examined the alkanethiol adsorption behavior on the modified titanium surfaces by using an electrochemical quartz crystal nanobalance(EQCN) developed in our laboratory. The resolution range of the constructed EQCN was about 0.1 ng. We were able to investigate precisely the mass change ratio on the surfaces versus immersion time. We examined the formation of SAMs(Self-assembled Monolayers) by alkanethiol on three working electrode surfaces(Au coated Ti, pure Ti and TiO₂). The mass changes on the Au/Ti and pure Ti surfaces were about 31 ng/cm² for 500 sec reaction time and 21 ng/cm² for 400 sec, respectively. On the other hand, the TiO₂ surface was substantially unchanged, indicating no formation of SAMs. The frequency variation on the TiO₂ surface was less than ±1 Hz for 400 sec reaction time. It is expected that Au and pure Ti surface may be modified into a functional bioactive surface by using alkanethiol.
- R/P.20** STAINLESS STEEL SURFACE MODIFICATION IN GROWING CELL CULTURES
M. Vinnichenko(a), Th. Chevolleau(b), M. Pham(b), L. Poperenko(a), and M.F. Maitz(b), (a)Kyiv Taras Shevchenko University, Kyiv 01033, Ukraine, (b)Forschungszentrum Rossendorf, Dresden 01314, Germany
Surface alteration of materials in biological systems is an important problem in implantology. Austenitic stainless steel 316L surface modification due to incubation in growing cell cultures (L929, polymorphonuclear neutrophils, SAOS-2) and cell culture media with fetal bovine serum (DMEM and a-MEM) as control has been studied. The modified surfaces were probed in comparison with untreated ones by means of spectroscopic ellipsometry, X-ray photoelectron spectroscopy (XPS) and atomic force microscopy. XPS showed the appearance of the peak of bonded nitrogen at 400.5 eV characteristic for adsorbed proteins on the surface for each type of cells and for the cell-free medium. Migration of Ni into the adsorbed layer was observed in all cases for samples after the cell cultures. The protein layer thickness was ellipsometrically determined to be within 2.5-6.0 nm for all treated samples with parameterization of its optical constants in Cauchy approach. The study showed that for such biological treatments of the stainless steel the protein adsorption is the dominating process in the first two weeks, which could play a role in the process of corrosion by complex forming properties with metal ions.
- R/P.21** STUDY OF THE REGENERATION OF CILIA IN CILIATE TETRAHYMENA BY ATOMIC FORCE MICROSCOPY
L.V. Melo(a), C. Casalou(b), S. Nolasco(b), A.C. Seixas(b), P. Brogueira(a) and H. Soares(b), (a)Physics Department, Instituto Superior Tecnico, Av. Rovisco Pais, 1049-001 Lisboa, Portugal, (2)Instituto Gulbenkian de Ciencia, Oeiras, Portugal
The ciliate Tetrahymena is an unicellular eukaryote having their bodies covered by cilia whose structure is identical to that found in the cilia of higher eukaryote ciliated tissues (for example, oviducts and respiratory tract). Cilia regeneration is an attractive model system to study the induced expression and regulation of the set of genes involved in cilia structure and assembly. Initially the cells had their cilia removed mechanically. The cells then regenerated their cilia, recovering mobility. The regeneration status was studied after different times. We report for the first time the direct observation of reciliating cells at a submicron scale by Atomic Force Microscopy(AFM) in atmospheric Tapping ModeTM. The AFM measures on the deformable surface of cell bodies. The cells were fixed onto nitrocellulose or glass. The cells surface show strong topography and presents sticky culture medium residues. The measurement performance depends from a tradeoff between culture medium richness (resulting in smoother body cells) and the amount of sticky residues affecting the AFM measurements. Our study shows that at 0 min of cilia recovery of the cilia organizing structures are disassembled and progressively regenerate and acquiring the standard structure. After 90 minutes of reciliating time most of the cilia are already regenerated which correlates with the motility of the cells. These results show that AFM is a promising technique for studying cell structure morphology.
- R/P.22** MORPHOLOGICAL AND ANIMAL STUDY OF TITANIUM DETAL IMPLANT SURFACE INDUCED BY BLASTING AND HIGH INTENSITY PULSED Nd-GLASS LASER
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Some earlier results showed that the osseointegration of Ti surface blasted with Al₂O₃ powder of 25 micrometer size is better than those were machined and blasted with Al₂O₃ powder of 250 micrometer size (1). Our earlier data showed that the laser irradiation with Nd-glass pulsed laser can induce surface morphology in 1-50 micrometer size range on as machined Ti surface which maybe promising for the improvement of the osseointegration. The combination of the blasting with the laser irradiation would be one another step to determine the morphology optimal for the osseointegration because the pulsed laser irradiation increases the density of the micrometer sized surface elements.
Machined dental implants of Ti with commercial purity were blasted with Al₂O₃ powder with 250 micrometer size. This surface was irradiated in vacuum with Nd-glass pulsed laser maximum at 3 J energy. The morphology of this surface was investigated by scanning electronmicroscopy.
The osseointegration was determined by the measurement of the removal torque at the rabbit experiments. The results were referred to the as machined surface of Ti dental implant. The blasting slightly increased the removal torque, but the irradiation with the high laser intensity increased the removal torque by factor of 1.5 compared to the reference.
1. A. Wennerberg, T. Albergsson, B. Andersson
J. Materials Science. Materials in Medicine. 6 (1995) 302-309.
- R/P.23** TRIBOCHEMICAL STRUCTURING AND COATING OF IMPLANT METAL SURFACES WITH TITANIUMOXIDE- AND HYDROXYAPATITE-LAYERS
U. Gbureck, R. Thull, Chair of Functional Materials in Medicine and Dentistry, University of Wuerzburg, Germany
Sandblasting of metal surfaces of implants with corundum leads to a significant alumina contamination with a coverage degree of 30 - 40%. New sandblasting materials to avoid contamination of titanium- and CoCr-alloys are titaniumoxide- and hydroxyapatite-corundum composite ceramics. Treated and untreated surfaces were analyzed by energy dispersive X-ray- (EDX) and X-ray diffraction-analysis (XRD) with regard to chemical composition. The surface morphology and roughness were measured by means of scanning electron microscopy (SEM) and atomic force microscopy (AFM). The results show a decrease of alumina contamination of the surface below a coverage of 15%. Besides activation and roughening tightly adhered coatings of titaniumoxide and hydroxyapatite were obtained on the metal surface. The concentration of the coating material close to the metal surface show a peak value of about 40 - 60% with a gradient shaped profile up to a depth of 5 - 6 µm. For structuring fixation elements in direct contact with bone the new blasting material is urgently suggested.

SYMPOSIUM R

Session V

- R-V.1** 15:00 BIOMIMETIC GROWTH OF HYDROXYAPATITE NANOCRYSTALS AND THEIR INFLUENCE ON BONE TISSUE CELL BEHAVIOUR
Norberto Roveri, University of Bologna, Italy
- R-V.2** 15:45 NANOTOPOGRAPHY AND ADHESION –CELLS AND PARTICLES
Adam Curtis, University of Glasgow, U.K.
- 16:30 **BREAK**
- R-V.3** 17:00 POLYFUNCTIONAL FILM COATINGS FOR MEDICAL USE
Galina U. Ostrovidova, Saint-Petersburg Technological University, Russia
At present the research and creation of materials for medical use are causing a great interest. Special attention is paid to such properties of materials as structure and composition of the surface, their biocompatibility and antimicrobial activity, as well as biodegradation [1,2]. Such properties can be passed to material by electroformation on its surface of polyinolecular structures, which possess directed physiological action. Creation of such polymolecular structures in the electric field is permitting to regulate their width and composition on the surface of the material to be modified[3].
As a polymeric matrix for the formation of the coatings and location of biological active substances polyvinyl alcohol (PVA), polyvinylpyrrolidon (PUP) and lactic acid were used. A proteolytic enzyme - trypsin and antibiotic - kanamycin sulfate were added to the composition during formation of coatings, in order to increase their biocompatibility and to provide them with antimicrobial activity. Under the influence of an infialow frequency electric field (signal regime of “meander” type) all the components are involving in the process of formation of polymolecular system. Due to the canting of the process on the negatively charged surface of support and positive charge of the molecules of trypsin and kanamycin, it is possible to control the amount of immobilized biologically active substances, and hence their activity[4]. During the biological experiments was proved the antimicrobial activity of obtained coatings. It was established, that the growth oppression zone oftest-microorganisms for used compositions was up to 80 mm.
The coatings formed from PVA and PVA-PUP composition, are possessing the most prolonged antimicrobial action (not less then 9 days). The addition of lactic acid to the working composition is valuably influencing the biodegradation rate. The period of the existence of such structures with a content of lactic acid of 70-80% decreased to several hours.
Different water-soluble polymers were used for formation of polyfunctional film coatings in electric field on various materials and items for medicine.
Support of scientific programme- INTAS-FOOD 01-876
[1] Julio San Roman. Desing and application of polymeric acrilic hydrogels for vascular surgery// Mat. 5-th European Polymer Federation Symposium on polymeric Materials. Basel, Switzerland. October 9-12, 1994, p. 171-173.
[2] Ioannis V.Yannas. Tissue and Matrix Regeneration using Analogs of Extracellular Matrix//See above.p. 159-164,
[3] Ostrovidova G.U., Makeev AV. and Zamyslov E.V. Electroformation of thromboresistant coftings based on polyvinyl alcohol//Russian Journal of Applied Chemistry. New York. Vol. 73,2000, N 5,p.881-884.
[4] Belikov V.G. Pharmaceutical chemistry.Moscow,Visshaya shkola,1993, 432p. (in fuss.).
- R-V.4** 17:20 ADHESION OF MICROSPHERES ON NANO-ENGINEERED SURFACES IN A PARALLEL PLATE FLOW CHAMBER
Elena Martines, University of Glasgow, U.K.
Cell adhesion under flow conditions has been extensively studied because of its important role e.g. in the immune and developmental system[1].
Since topographic cues have been shown to influence cell adhesion to surfaces [2,3], our aim is to elucidate the mechanisms for the changes in adhesion onto nanometrically engineered surfaces.
Because of the complicated receptor-ligand driven nature of cell adhesion, we started by investigating the adsorption/desorption rate of spherical beads (2.8 microns diameter) in a laminar flow over a plane away of nanopillars (80 nm height, 300 nm centre-to-centre).
The sphere-to-surface collision efficiency was measured, and compared to the sphere-to- surface collision efficiency on a smooth surface of the same material (PMMA). Finally, data analysis and the experimental procedure are detailed and discussed.
[1] Anne Pierres et al., J. Imm. Meth., 1996
[2] Man Dalby et al., Biomat., in press
[3] Adam Curtis et al., Biophys. Che., 2001
- R-V.5** 17:40 PLASMA PROCESSES FOR TISSUE ENGINEERING: MICRO-PATTERNED PEO-LIKE NON FOULING COATINGS
R. d'Agostino, E. Sardella, R. Gristina, P. Favia, Department of Chemistry, University of Bari, Istituto di Metodologie Inorganiche e dei Plasmi, CNR, via Orabona 4, 70126 Bari, Italy
Low pressure plasma processes of interest for tissue engineering and other biomedical applications have been utilised for patterning polymers in the micron scale. The surface of polystyrene and other substrates has been patterned with micro domains characterized by different chemical features, each one able to induce different adhesion, spreading and growth behaviour of cells. Copper grids and laser-cut kapton “physical masks” have been utilized to transfer the patterns. Coatings plasma deposited from acrylic acid and nitrogen containing groups plasma-grafted from ammonia have been utilized to develop cell adhesive tracks at the surface of the substrates; plasma-deposited polyethyleneoxide(PEO)-like coatings, instead, have been utilized for developing non fouling wider areas. Micro patterned substrates have been utilized in cell growth experiments, where they have shown marked ability in driving cell alignment and growth along definite directions.

SYMPOSIUM R

R-V.6

18:00

INFLUENCE OF SURFACE PATTERNING AND/OR STERILIZATION ON THE HEMOCOMPATIBILITY OF POLYCAPROLACTONES

Marigo Stavridi, Yannis Missirlis, Department of Mechanical Engineering, Biomedical Engineering Laboratory, University of Patras, Greece

Our objective was to evaluate human platelet adhesion, activation and blood coagulation on flat, nanostructured, sterilized or not sterilized membranes of polycaprolactones under static conditions. Fresh blood was obtained from three healthy volunteers who were not under medication for more than one week. Anticoagulant was used in all cases. The blood was centrifuged at 300 g for 10 min immediately after collection to obtain platelet rich plasma (PRP). Platelet poor plasma (PPP) was prepared by a further centrifugation at 3000 g for 15 min. Polycaprolactone-PCL^{*} samples were nanopatterned on the surface to have linear rectangular grooves, with width of 450nm. Some of the samples were sterilized using electron beam sterilization with dose of 2.5 Mrad.

Membrane samples were fixed in Teflon chambers of 10 mm well diameter. 1ml of PPP or 1ml of PRP was added to each well. After 30 min contact time, the PPP was collected and the nAPTT was determined by plasma recalcification technique using a C.K. PREST assay in a ST4 coagulometer, to measure the coagulation time. PRP was also collected after 30 min contact time and platelet counts were made with a Coulter Counter. PRP and PPP without membrane contact were used as control, whereas regular glass was used as negative control.

Our results indicate that contact of blood with all PCL surfaces cause high levels of adhesion. This can also be observed by scanning with an Atomic Force Microscope (AFM). The coagulation time seems to be affected by the nanopatterning and the sterilization procedure. Blood in contact with patterned surfaces coagulate later than blood in unpatterned surfaces. The coagulation time improves after sterilization. Platelet activation which results from the interactions with artificial surfaces leads to the release of the contents of the α -granules, especially α -TG and platelet factor4 (PF4). We used the Asserachrom α -TG enzyme immunoassay procedure for the quantitative determination of α -TG. Polycaprolactone surfaces seem to trigger high levels of platelet activation. Similar experiments under dynamic flow conditions are currently in progress.

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