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The lectures and abstracts from ***1st Workshop on Artificial Organs, Biomaterials and Tissue Engineering*** have been revised and evaluated by a scientific committee

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L1.
NON-INVASIVE EVALUATION OF CARDIAC VALVES PROSTHESES
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Blood flow in the heart chambers is one of the most interesting phenomena occurring in the cardiovascular system. Heart valves operating in passive mode regulate blood entering and leaving the ventricles, allowing proper filling and ejecting of blood. Backflow is minimised in the valve-closing phase and no pressure drop occurs in the opening phase. By working this way, haemolysis and thrombosis are neglected in the closing phase, and the flow produced during the opening phase does not impose harmful conditions to the blood cell. Problems arise when it is necessary to replace natural valves by artificial devices. Flow perturbations produced by the artificial heart valve implantation in the heart ventricle must be minimised in order to allow proper systemic tolerance. Since it is a complex problem to modelling – the flow occurring in the aorta root, for example, is in the transition from laminar to turbulent and, therefore, there is no mathematical model to it – experimental techniques are employed to preview the valve prosthesis performance *in vivo*. The implantation of artificial heart valve imposes hemodynamic changes that may be tolerated or not by the recipient circulatory system. Long term performance of tissue valves (those produced from modified biological tissue) is severely affected by calcification and by material degradation. Blood flow regime passing through the prostheses induces loads in the valves leaflets that may expose chemically reactive surfaces (the most common consequence is the leaflet calcification) or even produces initial sites for tearing the valve leaflet. The location of calcium deposition sites in the valve leaflets is related to the collagen exposed areas (material failure by stress) and to the local blood flow pattern (facilitating the transport of chemical species presents in the blood to the artificial tissue). In this context, the knowledge of the fluid flow pattern upstream and downstream the valve prostheses is a useful information to understand the calcification process occurring in tissue valves and may be employed in the design of new artificial heart valves. In the last decades, many authors have been working with flow visualization and measuring the flow field passing through biological and tissue heart valves. Hot Wire Anemometer (HWA), Laser Doppler Anemometer (LDA) and Particle Image Velocimeter (PIV) are the most employed techniques to perform flow field measurements. Recently, it has been described the methodology of performing the measurements of the flow passing through artificial heart valves using PIV 3D. This methodology opens new possibilities of analysing the flow upstream and downstream valve prosthesis, at the same time makes it possible to observe flow pattern in stereoscopic view.

L2.
BIOCERAMIC COATINGS
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Tissue response to biomaterials goes from the near inert to high chemical reactivity levels. The insertion of a biomaterial in the human body causes an inflammatory response, even though it can be a minimal one. Biocompatible metals and their oxides are the basis of the so called bioinert biomaterials, once implant-host tissue direct contact is observed, with thin portions of fibrous tissue. Inert surfaces are required in temporary implants such as bone fixation devices.
The project based on biofunctionality enrols surfaces that foster specific events cascades. In this direction, the so called bioactive materials are those capable of exchanging ions with body fluids, unchaining a series of biochemical reactions. In the case of bone implants, these reactions involve chemical bonding of the implant to bone tissue. Bioactive bonding is a result of the spontaneous precipitation of an apatite layer similar to bone apatite ("bonelike apatite"), from human body plasma, as well as extracellular matrix apatite mineralisation produced by osteoblastic cells which are in activity on the tissue-implant interface.
The establishment of the concept of bioactivity agrees with the developments achieved in calcium phosphate and bioactive glasses syntheses and *in vivo* and *in vitro* assessment. Fundamental studies allowed the development of new materials as well as the understanding of the mechanisms that lead to tissue responses. Bioactive bioceramics turned to be used as an alloplastic alternative for bone graft therapies. Additionally, bioceramic coatings on metals associated mechanical requirements from metals and alloys to chemical reactivity of bioactive materials.
Bioactive coatings on metals and polymers will be presented. Coatings consist of monetite, $CaHPO_4$, hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ and niobo-phosphate glasses. The coatings on metals aim to promote bioactive bonding between the metallic implant and bone tissue. The coatings on polymers consist of monetite deposition and further conversion to hydroxyapatite on porous polymeric scaffolds. This technique allows the production of porous and interconnected structures to be used as bone grafts as well as in tissue engineering.

L3.

BIOACTIVE GLASS AND HYBRID SCAFFOLDS FOR TISSUE ENGINEERING

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Tissue engineering is an important technique for regenerating diseased or damaged tissues. Tissue engineering of hard tissues is the main focus of the studies developed in our group. A highly porous artificial extracellular matrix or scaffold is required to accommodate cells and guide their growth and tissue regeneration in three dimensions. The choice of scaffolding material is crucial to the success of the technique. Bioactive glasses are an option as scaffold material for bone tissue engineering owing to their recognized osteoconductive and osteoinductive properties and controllable degradation rate. Resorbable 3D macroporous bioactive scaffolds have been produced by foaming sol–gel derived bioactive glasses with the aid of a surfactant. The preparation of bioactive glass with controllable structure and properties using the sol-gel route and the effects of processing variables on the structure and properties of the obtained bioactive glass foams were studied. The foams present high porosity varying from 60-90% and exhibit a hierarchical structure, with interconnected macropores(10–600?mm) and mesopores(2–50 nm). *In vitro* cell studies show an increase in osteoblast proliferation and collagen production in the presence of bioactive glass foams and the stimulation of the formation and mineralization of bone nodules, showing the potential of the foams as scaffold for bone tissue engineering. *In vivo* studies using bioactive glass foams associated with platelets rich plasma (PRP) in bone defects showed an increased bone formation and more mature bone formed when PRP was associated with the foams. The sol-gel method was also applied to produce bioactive glass/polyvinyl alcohol (PVA) hybrid scaffolds with improved mechanical properties. The degree of hydrolysis of PVA, concentration of PVA solution and different PVA-bioactive glass composition ratios affect the synthesis procedure. Synthesis parameters must be very well combined in order to allow foaming and gelation. The formation of the hybrid materials lead to an increase in strength with the additional advantage that this approach also leads to an increase in strain at failure and toughness. The hybrids obtained were submitted to neutralization procedure to control the toxicity. Neutralization methods used are effective in increasing cell viability levels. Hybrid samples were tested in osteoblast culture to evaluate adhesion and proliferation. Samples were also implanted subcutaneously in the dorsal region of adult rats. The hybrid 50% PVA/bioactive glass foam was chosen as the best scaffold in the composition range studied and it is a promising material for bone repair, providing a good environment for the adhesion and proliferation of osteoblasts *in vitro*. Concerning the *in vivo* studies the foreign body reaction was moderate and the presence of osteoid indicated bone matrix formation. Another hard tissue focused in the group is the cementum, a mineralized tissue lining the surface of tooth root that provides attachment of periodontal ligament to root and surrounding alveolar bone. Researches aimed toward understanding mechanisms involved in the formation of root cementum have been hampered by an inability to isolate and culture cells required in cementum production, i.e., cementoblasts. Cementoblasts were obtained from the root surface of extracted Wistar rat molars. Three cell populations derived from rat molar periodontal ligament were obtained. Analysis of RT-PCR confirmed the expression of F spondin, a possible cementoblast marker, in the isolated cells. Expression at protein level was confirmed by western blot and immunofluorescence using specific antibodies for F-Spondin. In order to evaluate the potential for the regeneration of cementum by tissue engineering, the interaction of cementoblast cells with chitosan membranes was investigated. The results demonstrate the possibility of isolation and characterization of rat cementoblast cell line and its use as a model for investigating cementoblast behavior. The chitosan membranes used in this study need further changes for application in cementum regeneration.

Key words: *Bioactive glass, hybrids, tissue engineering, bone regeneration, sol-gel*
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L4.

SCAFFOLDS CONSTRUCTION AND TISSUE ENGINEERING BY ADDITIVE FABRICATION

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The need for tissue and organ donors is a worldwide public and socio-economic challenge that grows everyday. Implanted organs and tissues are used to reestablish totally or partially biological functions due to diseases, traumas, or any malformation. When possible, the autologous transplantation of tissue is preferable to prevent cross contamination. In the case of unique organs the obit of donors has to be certified, besides high costs and some ethical and biological issues.

The reconstruction or substitution of parts of the human body date back to the last 3,000 years as have been shown by archeological artifacts as trepanned skulls or bones and teeth with some kind of implant. Moreover, some biological evidences of self regeneration like a tail in gecko, or even human organs like livers inspired concepts today known as "tissue engineering".

This new area promises to mimic, develop and apply tissue for increasing tissue regeneration and organs substitution. Tissue engineering is typically multidisciplinary involving materials science, biology, engineering, and clinical, among many others.

In 2002, the WTEC Panel Report on Tissue Engineering Research provided by International Technology Research Institute defined the expression "tissue engineering". This definition corroborated that coined by Dr. Skalak and Dr. Fox in 1988 in a National Science Foundation – NSF Workshop: "the application of principles and methods of engineering and life sciences to obtain a fundamental understanding of structure-function relationships in novel and pathological mammalian tissues and the development of biological substitutes to restore, maintain, or improve [tissue] function".

A decisive contribution for the establishment of tissue engineering as a new scientific area was made by the four Vacanti brothers, in special Joseph and Charles. In 1997, Joseph and Charles Vacanti in association with other researchers published in the Plastic and Reconstructive Surgery an article showing the viability to create tissues by means of synthetic materials and living cells. The experiment consisted of a biodegradable polymer (polyglycolic acid) device, in the form of a human ear, seeded with bovine chondrocytes and implanted on back of athymic (nude) mice. Their experiment is considered a historic watershed for tissue engineering arising hope for many people, in one side, and criticisms, on the other side.

The enablers for tissue engineering are essentially biomaterials, cellular biology, bioreactors, and clinical development, described as follow: 1) biomaterials originated from synthetic or natural sources, responsible to actively support the cells growing and differentiation. The biomaterial can be three-dimensionally structured in the macro and micro levels to create these supports known as scaffolds that after seeded with cells are called constructs; 2) the cellular biology is responsible for the harvesting and cultivation of viable and adequate cells to be seeded in the scaffold as the living basis for tissue formation. Additionally, biomolecules can be introduced as a functional regulator and integrator of biomaterials and cells. The Bone Morphogenic Protein (BMP) is one of them inducing bone formation; 3) bioreactors play an important role to help mature tissue before implantation. Concepts as perfusion, to feed and withdraw subproducts of the cellular activity, and mechanical and electrical stimuli applied to cells, have to be considered; 4) Finally, clinical development that consists of good practices and therapies.

Besides these enablers it is also necessary an appropriate design of the scaffold. Unfortunately, the today's computational systems known as CAD (Computer-Aided Design), in use in the industry, are limited for this application. It expected in future dedicated CAD systems able to design complex organs in terms of anatomy, geometry, and materials (living or not). The parameters to be considered in the Scaffold´s design, among many, are the anatomy, porosity (dimension, interconnectivity, and geometry), load bearing, biomaterial´s degradation rate, and surface characteristics as roughness and treatment.

Like in product development, additive fabrication (well known as rapid prototyping) is growing very fast in the area of biofabrication of scaffolds and living tissues. The paradigm of additive fabrication is the direct construction of a physical solid, based on deposition of material layers. This procedure can reproduce physically any type of geometry in many different materials directly from a virtual 3D model in a computer. Today´s processes of additive fabrication are oriented to the manufacturing field reproducing replicas or dimensionally precise prototypes for design or functional purposes. Also, it is applied to the direct production of small series of a product, called rapid manufacturing. The same concept is being investigated worldwide to reproduce 3D tissues or organs directly from a layer-by-layer deposition of biomaterials and cells. The deposition of biomaterials in a 3D controlled form, following the rapid manufacturing concepts, is called direct scaffold production. Researchers believe that in the near future a controlled biomaterial and cell deposition with an adequate design can reproduce living organs and complexes tissues.

This seminar will present the modern concept of additive fabrication in the medical area and the requirements of this strategic area for human kind. Narrowing the gap of technologies, CTI is working in different fronts. Among others, CTI is integrating and coining new concepts such as BioCAD, which is an adequate CAD system for tissue engineering applications, investigating three-dimensional structuring of biomaterials, medical image processing software, and bioengineering analysis. These research fronts are developed in tight partnership with two research nets: *Red Iberoamericana de Biofabricación* - BioFAB which is supported by the Latinamerican Science & Technology Development Programme – CYTED, and the Brazilian National Institute for Science and Technology in Biofabrication (*INCT Biofabricação*) supported by National Counsel of Technological and Scientific Development - CNPq and The State of São Paulo Research Foundation - FAPESP in Brazil. Additionally, CTI has a vast experience in applied research and solutions using and developing medical image processing, rapid prototyping and bioengineering analysis with dozens of universities by supporting master and PhD programs, and also with more than a hundred public hospitals in solutions for customized prostheses and surgical planning of patients with big anomalies, especially in the craniofacial complex.

L5.
BIOMATERIALS: HISTORICAL AND FUTURE DEVELOPMENTS
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During the last decades considerable attention has been directed towards use of implants. A revolution in medical care began with the successful replacement of tissues using "biomaterials" as tissue substitutes. Biomaterial is defined as a material intended to interface with biological system to evaluate, treat, augment or replace any tissue, organ or function of the body. Biomaterials can be metals, polymers, ceramics, composites and natural materials. Implants made with biomaterials include orthopedic devices such as total knee and hip joint replacements, spinal implants, bone screws and plates; artificial heart valves and pacemakers; soft tissue implants such as injectable collagen or poly (methyl metacrylate) for soft tissue augmentation; and dental implants to replace teeth and bone tissues in the oral cavity. Biomaterials now play a major role in replacing or improving the function of body system. This revolution coincided with the increase in overall human survivability.
Although many implants exist, for the improvement of the quality of life it is a constant challenge to optimize them and to develop new ones. New researches into the synthesis and modification of biomaterials has been crucial for the area of "tissue engineering", in which tissue cells and biomaterials can be combined to create natural, living tissue replacements.
In this work are presented a review of development of biomaterial in the last 50 years and are pointed the recent developments and a forecast to the future for new biomaterials developments.

L6.
CERAMIC AND COMPOSITE MATERIALS FOR TISSUE ENGINEERING
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Tissue engineering is a new field joining several knowledge areas. In summary natural or artificial scaffolds are seeded with cells from patient and then re-inserted in the region of tissue lesion. Several ceramic and composite materials can be applied as scaffold. Calcium phosphate ceramics are widely used for bone graft or as scaffold. These ceramics can be doped with metals such as magnesium, strontium and zinc. Usually they were porous within a certain range of particle size. After physico-chemical characterization *in vitro* or *in vivo* tests can be carried out. For example, the quality and intensity of the tissue response to two synthetic granular hydroxyapatites implanted in critical defects in the skulls of rats were evaluated. No significant differences in either the quality or quantity of cells in the inflammatory infiltrate between the experimental groups, or in the degree of angiogenesis and cell proliferation. It was concluded that both grafts are biocompatible and that the physico-chemical differences of these biomaterials did not affect cellular response.
In other work we aim to evaluate the relative role of the calcium phosphate surface chemistry and surface topography on human osteoblast behavior. For this, highly dense phosphate ceramics (HA and â-TCP) presenting two distinct nano roughnesses were produced. Some samples were goldsputter coated in order to conveniently mask the surface chemical effects, without modification of the original roughness. Our results indicated that the nanotopography of the studied ceramics had no effect on the cellular adhesion (cell spreading, focal contacts and stress fibers formation). On the contrary, strong topographical effects were verified on cell proliferation and differentiation. Moreover, the phosphate chemistry was responsible for changes in adhesion, proliferation and cell differentiation. On TCP, it was shown that the main influent parameter was surface chemistry, which negatively affected the initial cell adhesion but positively affected the subsequent stage of proliferation and differentiation. On HA, the main influent parameter was surface topography, which increased cell differentiation but lowered proliferation.
A comparative study of *in vitro* bioactivity of hydroxyapatite (HA) and silicon doped hydroxyapatite (SiHA) has been carried out by immersion in a cell culture medium with or without fetal bovine serum during 14 days in static and dynamic conditions. A specific bioreactor was developed for the experiments in dynamic conditions. The two hydroxyapatite surfaces immersed in cell culture medium under dynamic conditions were found to be more probably covered by a new Mg enriched Ca-deficient apatite layer than surfaces immersed under static conditions. These results suggest that dynamic procedure and medium with serum macromolecules seems to be more adequate to predict the *in vivo* activity of bioceramics. Moreover, SiHA presented a higher capacity of protein adsorption. Hydroxyapatite (HA)-type I Collagen (Col) composite is a tissue-engineered bone graft which can act as a carrier or a template structure for cells or any other agents. The effect of collagen ratio on the scaffold structure and composition was analyzed. Scaffolds composed by HA/Col with different weight ratios (80:20; 50:50; 20:80, and 10:90) were produced by the precipitation method at pH 8-9, 37 °C and 6 hours of ripening. Using X ray diffraction data, the Rietveld structure refinement showed that the size of HA crystals along the c-axis direction (002) decreases significantly in the presence of collagen. Thus, the HA crystal shape turned from needle-like in pure HA, into spherical, in the 10:90 composite due to collagen fibrillogenesis. The homogeneity of the composite was significantly dependent on the amount of collagen in it. HA/Col 20/80 composite presented HA particles in a more homogenous way. Such a biocomposite was successfully produced in a rapid way and it is potentially useful for both small tissue repairs and engineering.
Cartilage is a tissue difficult to be regenerated after an injury because its avascular nature. One of main trouble shown by cartilage is the

osteochondral defects. An attempt to solve this problem is the development of heterogeneous scaffolds, composed of two distinct layers, but integrated to repair the cartilage and bone tissue. Then, the hydroxyapatite (HA) was mixed with a chitosan (Chit) solution in order to obtain the first layer with composition 30/70 wt% (HA/Chit). The second layer was obtained by piled up of a chitosan solution on the first layer. By electron microscopy it was possible to differentiate both superior and inferior regions. The presence of Ca and P ions were associated with small particles (around 10μm) in the upper layer (superior part of Fig. 1), which confirm the presence of hydroxyapatite. The methodology employed allows obtaining an heterogeneous composite structured in two distinct layers, each layer exhibiting different characteristics. As the upper layer is proposed to repair the bone and the second is supposed to repair the cartilage, the next step of this work will be to evaluate the potential of this material using *in vitro* tests.

MC1.

THE VERSATILITY OF POLYURETHANE CHEMISTRY: STRATEGIES TO DEVELOP BIOMEDICAL ELASTOMERS WITH ENHANCED PROPERTIES

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After over 50 years of use in many biomedical applications, polyurethanes remain a popular choice due to their exceptional biocompatibility, mechanical properties and versatility. Polyurethane chemistry dictates the physico-chemical, mechanical, and biological properties of the resulting material and can be exploited to prepare a variety of materials including segmented polyurethane elastomers (SPU), rigid thermosets, adhesives, and foams. SPU are one of the most bio- and blood-compatible materials known today. These elastomers are block copolymers formed through step-growth polymerization by reacting a monomer containing at least two isocyanate functional groups with another monomer containing at least two hydroxyl (alcohol) groups in the presence of a catalyst. Properties such as durability, elasticity, fatigue resistance, compliance, and tolerance in the body, are often associated with polyurethanes. The unique and versatile mechanical properties of SPU elastomers are directly related to their two-phase microstructure, with hard domains acting as physically reversible crosslinking points, and soft domains that provide flexibility. The biostable or biodegradable character of SPU can be tailored. Thus, prolonged implantation requires materials with hydrolylic stability and oxidative-resistant soft segments (e.g., polycarbonate- or silicone-based polyurethanes), in combination with antioxidants or other additives. Bulk and surface modifications via hydrophilic/hydrophobic balance or by attachment of biorecognizable groups can also enhance the acceptance and healing of the implants.

The mechanistic understanding gained in the pursuit of enhanced polyurethane biostability was recently applied to the development of a new class of bioresorbable materials. The development of bioresorbable and biocompatible SPU and polyurethane networks for temporary applications has received considerable interest in recent years. Optimal design of biodegradable polyurethane scaffolds should meet the following criteria: 1) biocompatibility and clearance of all degradation products with minimal inflammation, 2) independent control of biodegradation and mechanical properties, 3) system-responsive degradation. Depending on their mechanical properties, chemical composition and surface characteristics, degradable SPU can potentially be used in designing cardiovascular implants, drug delivery devices, non-adhesive barriers in trauma surgery, injectable augmentation materials and tissue adhesives. Nowadays, the applications of polyurethanes in tissue-organ regeneration scaffolds is an area of intensive research, and some examples are cardiovascular tissue engineering, artificial skin, nerve regeneration and musculoskeletal applications (articular cartilage repair, anterior cruciate ligament, knee joint meniscus, meniscal reconstruction, bone tissue engineering, smooth muscle cell constructs for contractile muscle). To date, most of the bioresorbable materials available for use in tissue engineering are hard, brittle substances best suited to bone and hard tissue applications. Several tissues that need to be replaced or regenerated, however, are soft tissues that require large elasticity. Thus, there is a need for synthetic degradable materials that exhibit the properties of elastic recoil and malleability.

Segmental modifications of polyurethanes can be used to generate a library of polymers with broad structural diversity and derived properties. Tailoring the soft segment to achieve controlled degradation is a more common design strategy. To this end, a number of polyurethanes have been synthesized with hydrolytically unstable functional groups in the polymer backbone, typically poly(lactic acid), poly(glycolic acid), poly(å-caprolactone) and its copolymers. The synthesis of bioresorbable SPU requires a change from diisocyanates historically used in biostable formulations. Aromatic diisocyanates were often chosen for biomedical applications such as pacemaker lead coverings due to their enhanced mechanical properties. However, concerns that the degradation of these diisocyanates (i.e. 4,4'-methylenediphenyl diisocyanate, MDI) could generate potentially carcinogenic compounds (i.e. dianiline derivatives) has limited their translation to biodegradable polymers. Therefore, these aromatic diisocyanates were replaced with aliphatic diisocyanates such as L-lysine ethyl (or methyl) ester diisocyanate, hydrogenated MDI and hexamethylene diisocyanate that are more likely to have non-toxic degradation products. When appropriately designed, chain extenders are able to impart specific properties to the material. In this way, chain extenders are also investigated to promote highly ordered microphase-separated hard domains or to enhance hard segment degradation. The incorporation of urea-containing compounds or aromatic groups may increase hard segment cohesion by bidentate hydrogen bonding interactions or by π-bond stacking, respectively. In addition, the presence of hydrolyzable or enzyme sensitive linkages may modulate the degradative behaviour of hard domains. Bioresorbable polyurethanes have been shown to support the ingrowth of cells and undergo controlled degradation to non-cytotoxic decomposition products. These features combined with the tunable biological, mechanical, and physicochemical properties make these new materials excellent candidates for tissue engineering scaffolds. This presentation summarizes the recent advances and strategies in the synthesis of polyurethane systems, and its different applications in the medical field.

Key words: *segmented polyurethanes, elastomers, biomedical devices, tissue engineering*

MC2.
TARGETED DELIVERY OF QUANTUM DOTS-LOADED LIPOSOMES
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The design of liposomal carriers for gene or drug delivery is an active field in current biomedical research. An important issue is the targeting of lipid-based carriers to specific cell types for which a therapeutic approach is intended. The combination of liposomes and imaging probes such as Quantum Dots (QDs) in a single nanoparticle offers an excellent tool for understanding the mechanisms of liposome uptake, required to improve the efficiency of gene or drug delivery.
In the present work, biotinylated lipid particles (BLPs) were loaded with a red emitting QD_{655} and surface coated with a green emitting QD_{525} tagged with the Epidermal Growth Factor (EGF) ligand. This dual-color approach was employed to monitor the specificity of BLPs binding and uptake in A431 cells from human epidermoid carcinoma over-expressing the EGF receptor (EGFR) by confocal fluorescence microscopy. BLPs were formulated with 1.4% and 2.7% of PEG-lipids composed by either a fusogenic or a pH-sensitive lipid to favor endosomal escape of the encapsulated QDs.
Particle analysis by transmission electron microscopy showed that BLPs exhibited a spherical shape with a mean diameter of 120 nm and 2 to 5 encapsulated QDs per lipid particle for all the lipid formulations tested. BLPs loaded with QD_{655} were then conjugated to preformed complexes of biotin-EGF-streptavidin-QD_{525} (EGF-QD_{525}). With this approach, colocalized red-green dots were expected to indicate specific uptake, whereas the sole detection of red fluorescence would reveal unspecific binding of non-targeted BLP-QD_{655}. After 1 h incubation at 37ºC of A431 cells with the BLPs, both QDs extensively colocalized and were distributed in clusters in the perinuclear region, indicating that targeted EGF-QD_{525}-BLP-QD_{655} particles were internalized. Negligible internalization as evidenced by a faint red fluorescence was detected for non-targeted BLP-QD_{655}. Furthermore, no targeting effect was evident in CHO (Chinese Hamster Ovary) cells and in WM983A melanoma cells, both devoid of EGF receptor or in the presence of free competing ligand, indicating that enhanced cell uptake is primarily mediated by EGF-EGFR interaction. Regardless of the lipid formulation tested, the clusters of colocalized QDs persisted over days in A431 cells, indicating the difficulty in releasing the encapsulated nanoparticles into the cytoplasm.
We concluded that the dual-color QD labeling strategy allows the immediate detection of targeted BLPs from the untargeted counterparts, as well as the presence of free EGF-QD_{525} complexes during live cell imaging. However, the lipid formulations tested are unable to facilitate the endosomal release of QD_{655}. From a therapeutic point of view, the specificity displayed by the targeted BLPs may provide a platform for testing the specific delivery of anti-tumorigenic drugs into tumor cells overexpressing the EGFR. This approach was also intended to elucidate the fate of lipid particles in real time, taking advantages of the photostability and bright fluorescence of QDs and to provide refined information about the still poorly understood trafficking processes and the subcellular barriers to gene or drug delivery.
Key words: *biotinylated lipid particles, quantum dots, epidermal growth factor, targeted delivery, confocal microscopy.*

MC3.
ADVANCES IN THE DEVELOPMENT OF AN EXTRACORPOREAL BIO-ARTIFICIAL LIVER
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Novel therapies are required for the treatment of acute liver failure. Bio-artificial liver (BAL) systems are under development to offer better clinical performance than ordinary therapies, because they should be able to replicate in part the critical functions of the natural organ.Our design consists in a cartridge composed of a parallel bundle of hollow fibers housed within an external casing which incorporates hepatic spheroids (cells assembled together with great physiological activity). The hollow fibers are those usually used for microfiltration, but in our case we synthesized pore-filled ones with polyAcrilamide hydrogel. Blood or plasma normally flows through the fiber lumens, and spheroids are in the extracapillary space.
The present work focuses on the design of membranes that allow the differential diffusion of plasma metabolites and proteins such as immunoglobulin (IgG). This design will improve catabolites removal and also reduce possible immune response and virus infection.
We also consider a design which an appropriate supply of oxygen to a huge amount of cells. We present a system to measure transport of oxygen (more precisely the volumetric liquid mass transfer coefficient: kLa)
Finally we make some concrete proposals for the optimal design of bio-hybrid systems consisting of cellular tissue on a hollow fiber scaffold.
Key words: *hydrogels, diffusion, membranes, radiation-induced polymerisation, kLa*

MC4.
TISSUE ENGINEERING APPLICATIONS

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Tissue Engineering is an emergent discipline receiving knowledge from different field of sciences, as medicine, chemistry, biology, material sciences, physic, and others. A commonly applied definition of tissue engineering, as state by Langer and Vacanti Is "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ" (Langer R, Vacanti JP. Tissue engineering. Science 1993; 260: 920). Tissue engineering has also been defined as the understanding the principles of tissue growth, and applying this to produce functional replacement tissue for clinical use (MacArthur B, Oreffo R. Bridging the gap. Nature 2005: 433: 19). . Scientific advances in biomaterials, biology of cells, stem cells, growth and differentiation factors, and biomimetic environments have created unique opportunities to fabricate tissues in the laboratory from combinations of engineered extracellular matrices ("scaffolds"), cells, and biologically active molecules. Among the major challenges now facing tissue engineering is the need for more complex functionality, as well as both functional and biomechanical stability in laboratory-grown tissues destined for transplantation. Tissue engineering is a field that covers a broad range of applications, as development of tissue replacements (i.e. cartilage, bone, skin, cardiovascular, blood vessels, bladder, dental tissue), bioartificial support devices (bioartificial liver assit devices), artificial organs (heart), etc.
Two examples of Tissue Engineering applications will be discussed in the presentation.

1. Skin substitute for wound healing therapy. Use of scaffolds with co-cultured dermal fibroblasts and epidermal keratinocytes for clinic applications
2. Development of a bioartificial assist device.

MC5.
INTRODUCTION TO BIOMATERIALS

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Since ancient times materials have participated in all aspects of human life, to the extent that the technological stages of human development received the name of the materials conquered at the time (bronze age, etc). Particularly, they sustained the great XX Century developments and surely will sustain those of the future. Biological materials exist since life appeared on earth and we have very recently begun to comprehend their complexity and applicability. They are ancient materials but of similar structure of those which permitted the most spectacular progress of modern technology. Biomaterials are synthetic materials used to replace part of a living system or to function in intimate contact with living tissue. They are formally defined as systemically and pharmacologically inert substances designed for implantation within or incorporation with living systems.The uses of biomaterials include replacement of a body part that has lost function due to disease or trauma, to assist in healing, to improve function and to correct abnormalities. The success of a biomaterial depends on material properties, design, biocompatibility and the technique used by the surgeon. Biocompatibility is the acceptance by surrounding tissues and by the body as a whole, being a function of localization, composition and shape of the implant. Failure may occur mainly by infection, loosening, fracture and/or wear. Materials employed are metals, polymers, ceramics and composites. All of them are characterized by a structure that determines its elastic properties and corrosion resistance. Some of the examples considered are: implants used in hip replacement, artificial aorta, aortic valve and dental materials, among others. Finally, fixators employed in osteosynthesis are treated here, starting with a historical and comparative analysis of different systems, detailing methods to determine properties and stability. It concludes with the description of an application to correct tibia head deviation that difficulties normal mobility.
Key words: *biomaterials, reliability, fixators.*

TE1.

IMMOBILIZATION OF HYBRIDOMA CELLS IN SILICATE MATRICES FOR MONOCLONAL ANTIBODY PRODUCTION

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The encapsulation of biosystems including antibodies, enzymes, bacteria, and mammalian cells is becoming popular in several fields such as building of biosensors, bioreactors and cell encapsulation for bioartificial organs. The sol-gel process is an inorganic polymerization taking place, in some cases, in mild conditions allowing the association of mineral phases with biological systems. These gel matrices enable prolonged use of cells in an optimum environment and may provide the conditions for high productivity.
In this work, we studied the effect of different parameters such as the concentration of different sol-gel precursors and immobilized cell density on the function of hybridoma cell viability and Mab production. When studying the effect of the concentration of sol-gel precursors (150-600 mM), a higher metabolic activity was observed in matrices containing the lowest silica concentration. However, when low silica concentrations are used the physical properties of the material change and they lack of mechanical strength. For that reason we decided to use 300 mM silica matrices. Cell morphology and membrane integrity were conserved as it was observed by optical and fluorescence microscopy. Cellular activity of immobilized cells was determined by the tetrazolium (MTT) assay and a LIVE/DEAD vitality fluorescent assay. Viability was 89.5% in tetrakis (2-hydroxyethyl) ortosilitate matrices and was similar to the values obtained in sodium silicate matrices. Hybridoma cells were not detected in the supernatant indicating matrices are efficient in retaining cells. Significant differences were observed in cellular activity when various cells densities were employed, being higher for lower cell densities when expressed on a per cell basis. Cellular activities observed for matrices containing $5x10^5$, $1x10^6$ and $1.5x10^6$ cells were 44%, 75% and 82% lower than those observed for matrices containing $2.5x10^5$ cells. The highest Mab concentration in the supernatant was found for $5x10^5$ immobilized cells. Antibody diffusion out of silicate gels was studied showing a slow released during the first 24 hs and rising in the next days. MAb production from immobilized cells was detected by ELISA for a period of 15 days. The production of Mab by immobilized cells was 10-20% of the concentration obtained with the same initial number of free cells. However, as immobilized cells are not able to grow inside matrices in the same level as free cells, at the end of the experiment, there were 70-80% less cells in silicate matrices than in suspension. Once we have accomplished the first step of our investigation achieving good viability results and preserving cellular ability to produce monoclonal antibodies, the next step is to optimized Mab yields.
Key words: *hybridoma, immobilization, sol-gel.*

TE2.

IN SITU FORMED BIODEGRADABLE SPONGE FOR EMBOLOTHERAPY

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Over the last years there has been an increasing interest in using endovascular therapies as a less invasive treatment option than conventional open procedures. Progress has been particularly made in embolization, which is a non-surgical, minimally-invasive procedure that aims to obliterate tumors, aneurysms and other vascular diseases. One of the most important factors to be considered is the effectiveness and safety of the embolic agent. The choice of the specific agent depends upon the clinical application. In the treatment of tumors, a slight permeable and not permanent occlusion is desirable. Arterial patency and progressive re-canalization of the via can be achieved using biodegradable material. In the present study, an endovascular *in situ* forming implant is explored for ultimate use in embolotherapy. After choosing biocompatible solvents, injectable solutions of a FDA approved synthetic biodegradable water-insoluble polymer as a precipitating agent upon contact with blood (P1) and of a water-soluble polymer (P2), and mixtures thereof, were used to this purpose. The performance of two formulations, P1 and P1+P2, was evaluated by carrying out *in vitro* and *in vivo* assays. *In vitro study*: Experiments were carried out using flexible ducts of appropriate diameter with controlled circulating flow rate by a peristaltic pump as a way of simulating an artificial endovascular system. The morphology and topology of the in situ formed implant were analyzed by scanning electron microscopy (SEM). *In vivo study*: The formulations were injected into the femoral artery of swine, and the *in situ* formation of the implant was followed by radiography with contrast media. On day 5 after injection, radiographies were done again to assess if there was bloodstream trough the implants. After sacrifice, a portion of the artery was used for histology. Results The implants formed *in vitro* were porous structures, like-sponges, as shown SEM micrographs. The size of pores was significantly higher in implants formed with P1+P2 than with P1. Radiographies revealed blood flow throughout the implant in both cases. The *in situ* formed system in the femoral artery of swine leads to the formation of thrombus. Histological analyses revealed that the thrombus of the animal injected with P1 was more organized and dense than that injected with P1+P2. This feature suggests that the formation of biodegradable sponges with larger pores will allow a faster re-canalization of the artery. Therefore, the addition of water-soluble polymers like P2 to P1 allows the formation of structures that appear to reabsorb faster, which will allow a prompt arterial flow recovery, process that is important, if not vital, in the embolotherapy procedure.
Key words: *endovascular implant, embolization*

TE3.
HIGHLY-POROUS ELECTROSPRAYED SCAFFOLDS OF COMPATIBILIZED PCL/PDIPF BLENDS FOR BONE TISSUE ENGINEERING

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Synthetic biodegradable polyesters are being extensively studied as biocompatible polymeric scaffolds for different tissue engineering applications. Poly(ε-caprolactone) (PCL) and poly(diisopropylfumarate) (PDIPF) have probed to support adhesion, growth and differentiation of two osteoblastic cell lines, mouse calvaria-derived MC3T3E1 cells and UMR106 rat osteosarcoma, suggesting that these polymers could be useful in bone tissue regeneration. The blend of these polymers can modulate the biodegradation rate. The compatibility between PCL and PDIPF can be improved by ultrasound. For these reason solutions of PCL/PDIPF blends were exposed to high-intensity ultrasound. Size exclusion chromatography demonstrated that chain scission produced macroradical formation. Moreover, sonochemically induced reactions led to interpolymer radical coupling and block copolymer formation, which acted as compatibilizer between PCL and PDIPF. The compatibilized PCL/PDIPF blend had better properties than the non-sonicated one. In this work, highly-porous scaffolds of the compatibilized blend were prepared by electrospinning. A polymer solution 4% wt/V in chloroform was delivered through a spinneret at a flow rate of 1.5 mL/h, using an applied voltage of 0.7 $kV.cm^{-1}$. The sample was collected on an aluminum foil covering the grounded aluminum collector. Experiments were performed during 1 h. The solution properties (solvent, concentration) and the processing conditions used in this work allowed the deposition of wet microspheres, which aggregated on contact with each other, instead of micro/nanofibers. The electrosprying generated a highly-porous interconnected structure formed by agglomerated microspheres. The obtained microspheres were irregularly shaped with approximately 5 μm in diameter and a narrow size distribution, as observed by scanning electron microscopy. Thus, the electrospraying technique could be a promising technique for the preparation of PCL/PDIPF scaffolds for bone tissue engineering. Additional experiments, varying solution concentration and processing parameters, could generate scaffolds with micro/nanofibrous morphology.

Key words: *poly(ε-caprolactone), poly(diisopropylfumarate), scaffolds, electrospraying, microspheres.*

TE4.
STUDY OF ε-POLICAPROLACTONE FOR SCAFFOLDS CONSTRUCTION BY RAPID PROTOTYPING

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Tissue engineering has been a great focus of research in the last years. These studies involve a multidisciplinary field of research, including, among many areas, biotecnologists, material and chemical engineers, and clinics. The main idea is to produce biological substitutes to replace damaged or missing organs or tissues. Therefore, scaffolds are expected to play an important role in allowing physicians to simultaneously reconstruct and regenerate damaged human tissue such as bone, cartilage, ligament and tendon. It is necessary a tight integration of biomaterials, cells, differentiation factors, and sometimes a place to mature the substitute, like bioreactors. One technology avaiable until now is the rapid prototyping or additive fabrication, a research tool for biomedical applications. This class of technology is based on technique of material deposition layer-by-layer to create parts with any complex geometry via Computer-Aided Design (CAD). Then, scaffolds with anatomical geometries and controlled pores in terms of size and shape can be produced. Among biomaterials, ε-caprolactone (ε-PCL), aliphatic polyester, is an interesting one for 3D scaffold prototyping for the reason that is biocompatible, biodegradable and can be easily processed. This work proposes the ε-PCL study as an adequate biomaterial to be used in rapid prototyping process to fabricate 3D scaffolds. We used an experimental platform - Fab@Home - with free and open-source hardware and software, using a Fused Deposition Modeling process. At first time, the ε-PCL was in form of powder and was transformed in filaments to be used at a heated deposition head developed and adapted for Fab@Home. Subsequently, under determined conditions, the filaments were extruded, producing the scaffolds.

TE5.
COMPUTATIONAL MODELING OF A TRI-LEAFLET MECHANICAL HEART VALVE

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A tri-leaflet mechanical heart valve was designed for getting the central flow and hemodynamic performance of a native valve. This tri-leaflet valve was designed using computational fluid dynamic modeling, solving the mathematical equations of the fluid flow assumed laminar and steady state flow. Following the premise of design a mechanical valve with three leaves, no obstruction in the center of the valve and peripheral pivots similar to a native valve, the optimum design was reached by progressive changes in the form. This design was compared with a standard bi-leaflet mechanical valve and a simplified model of a native valve. A right artery of 24 mm in diameter and aortic sinus whit rigid wall is assumed. For the comparison, the valves were located in this artery at 80 mm of the inlet and 200 mm of the outlet. Prosthetic valves of 24 mm in inner diameter were placed in suprananular position, with the leaves totally opened. The optimum design of the tri-leaflet valve shows that the effective surface area for flow across the valve is 84% of the total valve section; with that design the 98% of the flow is central. On the other hand, in the bi-leaflet valve the effective surface area is 79% of the total valve section, and the flow is divided in three channels: two laterals (76%) and one central (24%). For a stable flow of 5000 cm≈/min the pressure drop across the tri-leaflet valve is of 0.07 mmHg, considerably lower than the 0.17 mmHg for the bi-leaflet valve. As reference, the pressure drop calculated by our model of a native valve in the same conditions is of 0.045 mmHg. The shear stress for the tri-leaflet valve is 80% lower than for the bi-leaflet valve. It is concluded that this tri-leaflet mechanical valve design achieves a large central flow, which due to the large central area produce a reduced pressure drop and a lower shear stress than for the bi-leaflet valve and consequently a low consumption of energy and a lower risk for trombogenicity, slightly larger to the expected in a native valve.
Key words: *three leaves heart valve, mechanical heart valve, computational design, hemodynamic modeling, steady state flow.*

TE6.
NUMERICAL SOLUTION OF A THEORETICAL MODEL OF KNEE PROSTHESIS FOR THE DETERMINATION OF APPROPRIATE MATERIALS PARAMETERS

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Knee prostheses are currently the only solution for totally worn out knees. One of the major problems is the aseptic loosening of the implant, resulting from wear of ultra high molecular weigh polyethylene (UHMWPE). Science and technology are focused on the improvement of the mechanical response of UHMWPE and, at the same time, the development of new cartilage replacement materials. The aim of this work is to simulate the lubrication mechanism of both a knee prosthesis and a healthy knee in order to investigate the influence of the mechanical variables (pressure, film thickness and friction coefficient) on wear. To this end, we built a model of the lubrication between the femoral head and the tibial plateau, resulting in a set of nonlinear equations solved by means of a numerically technique based on the finite element method. The model takes into account the non-Newtonian characteristic of the synovial fluid, the concentration of the hyaluronic acid, as well as the visco-elasticity and exudation factor (porosity) of a hypothetical new replacement material. The numerical results show that UHMWPE has a very high elastic modulus to provide appropriated lubrication to the joint, leading to very large pressure values and very thin film thicknesses in the lubrication channel. However, a replacement material displaying visco-elastic characteristics showed thinner film thicknesses (of the order of the superficial roughness) even for a soft material, a very undesirable feature from the standpoint of wear. In this sense, the high rigidity of UHMWPE contributes to decrease the friction coefficient compared to softer materials. We also identify the mechanical features of a healthy cartilage responsible for the proper lubrication conditions and therefore low wear of natural joints, which should be employed as guidelines in the design of new engineered tissues.
Key words: *knee prosthesis, lubrication, mathematical model, finite element, engineered tissues.*

TE7.

COMPUTATIONAL SIMULATION TO PREDICT PROPERTIES OF A BIOMATERIAL-CELLS COMPLEX USING LATTICE MONTE CARLO METHOD

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A computer program, extremely portable and easy to install, was made with Visual Basic for Applications (VBA). It is executed in MS Excel, with graphics and animations, applying Lattice Monte Carlo method (LMC) to simulate and determine Biomaterial-cells assembly properties destined to implantation for tissue engineering. The LMC model addressing a phenomenological problem by the First Law of Fick [1] considering the interactions of cell integrin receptors with extracellular RGD ligands on biomaterial surface for the adhesion complexes formation. It was based on the work of Irvine *et al*. [2]. The behavior of receptors, initially placed randomly, is modified by probabilistic rules. If one of the cell receptors bonds to a ligand on the biomaterial, the cell immediately forms an adhesion focus in that zone. Thus, ligands should be located inside of clusters; otherwise the adhesion would be very low. For computational facility, square clusters were adopted. Concentrations from 800 to 1000 ligands by μm2, or greater for large clusters size (greater than 8 ligands by side), were necessary to arrive at the cell motility and a strong cell adhesion. This was validated with experimental results obtained by Maheshwari *et al*. [3]. The conclusions were that the same ligand-receptor complexes percentage on the biomaterial can be obtained applying a low density of Ligands with small clusters size or applying a high density with large clusters size. However, this last alternative permits to arrive at the saturation level (all receptors occupied). The need to reach saturation level (greater than 2400 ligands by μm2 with large clusters size) is that it would achieve high cell speed penetration in the scaffold. It should be proved.

Keywords: *adhesion receptor clusters, ligated integrin, Monte Carlo lattice simulation.*

References:

[1] Belova I *et al* (2007). The Lattice Monte Carlo Method for Solving Phenomenological Mass and Heat Transport Problems. Diffusion Fundamentals 4:15.1–15.23. Online. Available: http://www.uni-leipzig.de/diffusion/journal/pdf/volume4/diff_fund_4(2007)15.pdf.
[2] Irvine J *et al* (2002). Simulations of Cell-Surface Integrin Binding to Nanoscale-Clustered Adhesion Ligands. Biophysical Journal Volume 82: 120-132.
[3] Maheshwari G *et al* (2000). Cell adhesion and motility depend on nanoscale RGD clustering. Journal of Cell Science. p. 1677-1686.

TE8.

EVALUATION OF THE AMMONIA DETOXIFICATION EFFICIENCY OF RAT HEPATIC MICROORGANS USED AS THE BIOLOGICAL COMPONENT OF A BIOARTIFICIAL LIVER: A COMPARISON WITH THE PERFORMANCE OF ISOLATED HEPATOCYTES

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In previous works done by our research team, it has been assessed that both hepatic microorgans (HMOs) and isolated hepatocytes have the capacity to detoxify an ammonia overload in a normothermic reoxygenation system (NRS: 37°C, Krebs-Henseleit, 95% O_2:5% CO_2, 120 min). In addition, the ammonia detoxification capacity of isolated hepatocytes has been tested in a minibioreactor (MBR) suitable as bioartificial liver (BAL), developed in our laboratory. The MBR offers a biological compartment (Cb) separated from the plasmatic compartment (Cp) by commercially available hollow fibers. Blood circulates through the hollow fibers at a flow of 9 mL/min. Until now, only isolated hepatocytes have been used as biological component on this MBR.

The objective of this work was to determine the ammonia detoxification efficiency of rat HMOs used as an alternative biological component in the described MBR.

The HMOs were manually cut from Wistar rat livers into slices of 432±36 μm thickness, n=10. After a preincubation period (37ºC, Krebs-Henseleit, 95% O_2:5% CO_2), the HMOs were loaded into the Cb and an ammonia overload was administered to the blood used in order to obtain a final concentration of 0.95 ± 0.04 mM. Blood samples and samples from the Cb were taken after 60 and 120 min of perfusion.

The ammonia detoxification capacity obtained for HMOs in the MBR is shown in the following table, compared with HMOs in the NRS and isolated hepatocytes on both systems:

	Ammonia detoxification capacity (μmol/g wet tissue)			
Time (min)	**Hepatocytes NRS (n=3)**	**HMOs NRS (n=3)**	**Hepatocytes MBR (n=3)**	**HMOs MBR (n=3)**
60	21.0 ± 6.9	11.3 ± 1.3*	12.5 ± 1.8	2.6 ± 0.8#
120	25.5 ± 3.6	17.0 ± 3.3	18.6 ± 4.9	6.8 ± 1.2&

128 ∞ 10^6 hepatocytes = 1 g of liver

In all cases the ammonia overload was approximately 1 mM; * indicates different from Hepatocytes NRS, # indicates different from hepatocytes NRS and hepatocytes MBR, and & indicates different from all the other groups, ANOVA, $p < 0,05$.

Based on these results, we can conclude that:

A) After 60 min, the MOHs are less efficient to detoxify an ammonia overload than hepatocytes in both system.
B) However, the MOHs in the NRS show similar values to hepatocytes after 120 min of incubation.
C) On the other hand, the HMOs in the MBR were not capable of reaching the other groups performance. Further studies will be done to establish the best ammonia detoxification conditions for HMOs, in order to use them as biological component of a bioartificial liver

BM1.

DEVELOPMENT OF A SYNTHETIC HYDROXYAPATITE BIOCERAMIC MATERIAL, A HISTOLOGICAL AND HISTOMORFOMETRIC EVALUATION

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The objective was to develop a synthetic bioceramic material composed of calcium phosphate (hydroxyapatite), which was characterized chemically and studied to determine it biocompatibility to be used as a synthetic bone substitute. The hydroxyapatite was obtained by a synthetic precipitation that used inorganic calcium and phosphate salts which react in a water media. The product obtained was filtered and treated in a furnace at high temperature (>1100ºC) following an established temperature curve. After the bioceramic was obtained it was milled in a ball mill with a grinding alumina media. The material was characterized with radiologic, microscopic, chemically and mineralogic methods. Once the hdroxyapatite was confirmed by al the previous methods, it was studied in a rat bone marrow model of bone repair at the tibia bone, to evaluate it biological osteogenic activity. The powder was passed through a mesh in order to select the particle size (between 200 to 400 µm). Holes of 1.5 mm in diameter were drilled in the diaphysis of the tibia bones of 20 rats. The rats were divided into 2 groups of 10. One group was sacrificed at 14 days post surgery, and the other at 30 days. The tibias were dried and treated with formaldehyde. The biological samples obtained from the rat bones were evaluated in two different groups: Group I (right tibia) was included in methyl methacrylate; and Group II (left tibia) was included in paraffin. The biological samples were evaluated by X-rays, and by both optical and scanning electronic microscopy. The material was opaque to X-rays. The microscopic analysis of the samples of Group 1 showed particles of 200 - 400 µm and also others smaller than 50 µm. The larger particles were surrounded by laminar neoformed bone which was then surrounded by new bone bridges. The microscopic analysis of the samples of Group 2 showed particles surrounded by bone tissue. No signs of inflammation were visible. At the hystomorphometric evaluation it was shown that 88 to 89% bone integration was achieved. At 30 days post-implantation a slight increase in the new bone area was observed compared with the 14 days post-implantation evaluation. The synthetic hydroxyapatite bioceramic material produced was biocompatible and its particles were integrated with laminar bone united by new bone bridges. No inflammatory reaction was observed. The new bone synthesis increased over the time. The material showed bone induction and it can be use as bone substitute.
Key words: *Bioceramics, hydroxyapatite, bone substitute, biomaterial, biocompatibility.*

BM2.

DILATOMETRIC EVALUATION OF INORGANIC MOLD MATERIALS FOR CASTING OF DENTAL ALLOYS

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Casting of non-precious metals for dental applications is an ongrowing field in dentistry. Metal restorations are more robust, biocompatible and yield a long lifetime. The costs involved on casting non-precious metal alloys are relatively low, but the production process is critical. Considering the thermal expansion of investment materials, differences between investment and metal may provide low accuracy castings. Therefore, accurate measurements of the thermal expansion of the investment material are crucial to achieve good casting products. Investment differences on composition, manipulation and condensation may result in clinically acceptable castings, which, however, may not fulfill ideal standards. Two types of investments were evaluated, using a high accuracy dilatometry test and observation of the topography of the investments by SEM. A metal matrix was designed for the production of 6 cylindrical investment samples for each investment, with 25mm length and 6mm diameter, for the dilatrometric test. Both commercial investments evaluated (R1 e R2) were manipulated and subjected to thermal cycling under the conditions recommended by the manufacturer. After the dilatometric test, each sample was superficially evaluated by the SEM with magnifications of 50x, 270x, 400x e 1000x. The 6 dilatograms obtained for each investment showed concordance on phase transformation peaks; however, differences were observed in the linear thermal expansion coefficients for the temperatures examined, in both investments. For the investment R1, final values of linear expansion coefficient between -0,1% e -0,6% were observed; and for R2, between +0,15% e -0,45%. This fact can be explained by differences in handling, compaction, batch number and storage time of the investments. In the result interaction, a different thermal behavior between R1 e R2 was observed. This fact can be explained by differences in the composition of the investments, evidencing positive and negative aspects of each investment. Photomicrographs showed differences between the topographies of both evaluated investments. The dilatograms and photomicrographic analysis are accurate tools for investigation of acceptable investment results in dentistry.
Key words: *biocompatibility, inorganic ceramic, investment, dental alloys*

BM3.

WEAR RESISTANCE OF TiO_2 THIN FILMS TO PROSTHETIC HEART VALVE

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Titanium dioxide (TiO_2) thin films have good haemocompatibility, which is required for covering mechanical heart valve prosthesis. In addition the films must have good wear resistance and adherence. In the present investigation the wear of TiO_2 thin films made by a sol-gel dip coating technique is studied. A rotating ball-on-flat machine was used in order to reproduce the movement of a pivot of a mechanical heart valve. Ethylenglycol was used as lubricant; and a 6 mm in diameter glass ball was used as counterface. A constant velocity of 7 rpm and a load of 1 N were chosen to perform the tests. Electronic microscopy and X-ray diffraction were used to characterize the coating and measures the anatase-to-rutile ratio, respectively. The influence of the following dip-coating process parameters was analyzed: the withdrawing velocity, the aging time and the temperature of the heat treatment and the number of oxide layers. The thickness of the films obtained ranged from 25 to 200 nm. The withdrawing velocity and aging time had an influence on the film thickness. On the other hand, the temperature of the heat treatment and the numbers of layers had more influence on the wear resistance than the withdrawing velocity and the aging time of the sol. Higher process temperature and more layers increased the wear resistance. The temperature has an influence on the crystal structures of the titanium dioxide; three different cases were observed: only anatase or rutile, and a mixture of both.

Key words: *titanium oxide, sol-gel dispersion, thin films, wear resistance*

BM4.

NEED FOR DEVELOPMENT OF NEW MATERIALS IN ORTHOSIS AND PROSTHESIS

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Currently Argentina depends almost exclusively on orthoprosthetics equipment purchased in other countries, with very high costs and, in many cases, are hardly suitable for the needs of people with disabilities in the context of activities and physical spaces in which unfold daily in our country, requiring adaptations of "home" that eventually end up reducing or eliminating the benefits for which they were originally designed. In our Institute we are designing and making prototypes of low-cost orthoprosthetics elements This work is twofold, first to develop products for easy access, manufacturing, maintenance and use by those in need, as an alternative to the provision of foreign elements. Secondly, through the transfer, we intend to collaborate with the development of national industry in orthosis and prosthesis, virtually nonexistent in this area today. These tasks necessarily require applied research that integrates different fields of knowledge. To this end, actions have been performed with various workshops, institutions and enterprises in order to produce prototypes, test them and then, once assured of quality results, to produce on a larger scale. Since the start of these activities in 2000, made many of its aims, including the training of human resources, a mechanical model of prehensile hand for teaching purposes, the formation and continued active participation in the subcommittee of "orthosis and prosthesis" in the IRAM. Have already been officially published 5 standards in the area, and others are in full development. We also performed the design, development, production and biomechanical analysis of a low cost single axis knee, which was subjected to experimental tests by specialists in biomechanics of CENARD. In 2007, we filed and publicly exposed the project products and results, reaching the final instance of the national selection INNOVAR, organized by the Ministry of Science and Technology. From the results we obtained in these few years, we believe it must move in the direction of new materials for the production of orthoprosthetics elements. We are convinced that it is essential the participation of the University Community, but also other health institutions. To achieve optimum results will be critical that the association has the purpose of a cooperative relationship of mutual support from the institutions that work together to develop a common project. Projecting into the future, we are sure that in a relatively short time, applied research on new materials for the production of orthoses and prostheses may generate patents, transfer agreements with industry, health centers, forming this way a social network that help meet the needs of a broad spectrum of society often ignored.

Key words: *new materials, orthosis, prosthesis, relationship university - institutions*

BM5.
BACTERIAL ADHESION TO INTRAUTERINE DEVICES (IUD)
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The use of intrauterine devices (IUDs) as a method of contraception is a widespread practice characterized by its reversibility, high effectiveness/cost relationship and long life.
The most used devices are those based on copper which are made of a thin copper wire with polymeric threads at the bottom. After the IUD implantation, the ends of these threads are left in the vagina outside the cervical canal. The contact of these threads with the bacterial flora of vagina may increase the risk of uterine infections and pelvic inflammation.
The aim of this work was to study Pseudomonas aeruginosa attachmet and spreading on the IUD threads. With this purpose the top of the threads were masked with an inert material and then the whole trreads were immersed in the bacterial culture for two hours. During this period, a biofilm was formed on the bare surface. Subsequently, the masks were removed and the samples were rinsed and placed in a sterile culture medium during different periods from 2.5 to 60 h. Microscopic observations of bacterial attachement on the threads were performed after staining with crystal violet. A marked surface roughness was evidenced on the polymeric substrata and bacteria were preferencially placed in the valleys of the ditches. Preliminary studies indicated that the top of the threads can be colonized by bacteria from the biofilm formed at the botton of the thread. Consequently, IUDs may facilitate the spreading of microorganisms from the vagina towards the cervical canal, increasing the risk of uterine infections.
Acknowledgements: *ANPCyT (PICT 06-621, PICT 05-33225, PICT 05-32906, PAE 22771), UNLP (Grants11/X425 y 11/I129), and CONICET.*
Key words: IUD, intrauterine device, uterine infection, biofilm, bacterial adhesion

BM6.
ANTIBACTERIAL EFFECT OF SILVER NANOPARTICLES ADSORBED ON TITANIUM SURFACES
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One of the most important issues in the failure of implants is the formation of biofilms of pathogenic bacteria that can lead to infection. For this reason, it is imperative to find alternative surface treatments to reduce the risk of infection and the consequent rejection of the implanted material. Due to growing bacterial resistance to antibiotics, the study of the antimicrobial activity of silver, whether in the form of Ag^+ ions or, more recently, in the form of nanoparticles, has taken up importance in the last years. In this sense, the use of certain silver nanomaterials to obtain a better effect against microorganisms is already being used to prevent colonization of bacteria in different materials such as biomedical prostheses, catheters, dental materials and instruments, and even human skin.
The aim of this study is to investigate the activity of silver nanoparticles adsorbed on titanium substrates against *Pseudomonas aeruginosa*. To this end, titanium substrates were modified with silver nanoparticles (AgNP) by immersion in solutions for 24 h. The AgNP- modified substrates were characterized by Atomic Force Microscopy (AFM), X- Ray Photoelectron Spectroscopy (XPS), and Scanning Electron Microscopy (SEM). Results show that the nanoparticles (which have a mean diameter of ≈ 8 nm, as observed by Transmission Electron Microscopy (TEM)) are formed by metallic Ag and adsorb on the Ti/ TiO_2 surfaces forming clusters 100- 300 nm in size.
Regarding the antimicrobial activity of the prepared surfaces, several tests were performed. First, to verify the effectiveness of the nanoparticles as antibacterial agents, AgNP- modified surfaces and filter paper disks soaked in AgNP solution were incubated in cultures of *P. aeruginosa* in Mueller Hinton agar. Results show in all cases inhibition halos around the modified substrates. To assess the morphology of the biofilms on the Ti/TiO_2 surfaces, AgNP- modified substrates (and AgNP- free substrates) exposed to *P. aeruginosa* cultures were observed by AFM. Results show a similar amount of bacteria on surfaces with and without AgNP treatment. To check the viability of bacteria adhering to the surface, assays were performed in which the substrates were exposed to cultures at different incubation times, rinsed with sterile water, and then immersed in solid or liquid sterile culture media. In solid media, it was found that the growth halo was much smaller in the case of the AgNP- modified substrates than for the respective controls. The samples incubated in sterile liquid media were imaged by epifluorescence microscopy by using the LIVE/ DEAD® Baclight™ kit, and showed reduced viability for the AgNP-modified substrates.
The results obtained show that silver nanoparticle solutions are effective to inhibit colonization of *P. aeruginosa* on titanium surfaces.
Key words: *biofilms, silver nanoparticles, titanium, P. aeruginosa.*

BM7.

BIOCOMPATIBLE AND ANTIBACTERIAL PROPERTIES OF TITANIA-AG COMPOSITE FILMS OBTAINED BY ELECTROPHORETIC DEPOSITION

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The development and characterisation of titania (TiO_2) films has been extensively studied and clinically applied for its well-known biocompatibility and biocide properties in tissue engineering. The titania is an attractive ceramic widely employed in hard tissue replacement surgery. It forms a bone-like apatite on its surface in a simulated body fluid (SBF). It´s normally applied as a coating on metallic substrates in order to combine the mechanical strength of metals with the excellent biological properties of this oxide.
In recent years, the use inorganic antibacterial materials are superior to those of organic antibacterial materials in durability, toxicity and selectivity. The usefulness of Ag as an antibacterial agent has been known and exhibits low toxicity towards humans' cells. In this investigation, it is studied the fabrication of the TiO_2-Ag nanocomposite films on metallic titanium substrates by electrophoretic deposition (EPD). The Ag nanoparticles (nAg) were directly formed on the surface of TiO_2 nanoparticles from nucleophilic reaction catalysed by alkalis. The advantages to form nAg on supporting titania is that allows the release of Ag in a controlled manner against time.
The production of the films for biomedical applications requires a well control of the deposition conditions because they will affect the microstructure and properties of the films. TiO_2-nAg coating on Ti substrate were obtained using aqueous suspensions of TiO_2-nAg nanocomposite and 4% of ethanol. Before EPD, the substrates were etched with sulphuric acid (H_2SO_4) in order to improve the adhesion of the films. The substrates were placed as the cathode in the EPD cell. The EPD coatings were sintered at 700 ºC in vacuum for 2 hr. The microstructure and composition of the composite coatings were analyzed and measured using transmission electron microscopy (TEM), scanning electron microscopy (SEM), X-ray diffraction (XRD) and electron dispersive scattering (EDS). The antibacterial activity of TiO_2-nAg coatings was examined using the zone of inhibition (ZOI) test for the Staphylococcus aureus.
The results obtained in this research showed that nAg are homogeneously formed on the surface of TiO_2 nanoparticles and that the films of TiO_2-nAg developed using EPD have high bonding strength to titanium substrate, excellent biocompatibility and antibacterial property and this enhance the bio-performance of the coating, and is thought to be a promising implant candidate in repairing or replacing the damaged bone tissues.
Key words: *antibacterial, titania, electrophoretic deposition.*

BM8.

IN VITRO BEHAVIOR OF A TRICALCIUM PHOSPHATE CEMENT FOR USE IN MEDICAL APPLICATIONS

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Tricalcium silicate is the major constituent of Portland cement and the responsible for their mechanical strength at early stages. In order to be used as and additive of conventional calcium phosphate cement (CPC), *in vitro* bioactivity of a calcium silicate cement (CSC) after soaking in simulated body fluid (SBF) for 14 days was study. The cement was obtained by mixing Ca_3SiO_5, obtained by sol-gel process, and a Na_2HPO_4 solution. The morphological and structural changes of the material before and after soaking were analyzed by X-ray diffraction (XRD) and scanning electron microscopy (SEM). Mechanical properties were measured with a Universal Testing Machine with a load measuring cell of 10 kN. The results showed the formation of a layer of a Hydroxyapatite (HA) on the CSC cement surface after soaking for 1h in SBF that became denser with the increase of soaking time. The porosity before and after immersion were very similar 40.76 ±2.31 and 41.56 ±1.5. There was not significance differences between the values of compressive strength before and after soaking in SBF and these values were higher than traditional CPC prepared in the same conditions. The study suggests that Ca_3SiO_5 would be an effective additive to improve the bioactivity and long term strength of conventional CPC.
Key words: *tricalcium silicate, calcium silicate cement, calcium phosphate cement, in vitro.*

BM9.

BIOCOMPATIBILITY OF ZIRCONIUM AS AN ALTERNATIVE BIOMATERIAL FOR PERMANENT IMPLANTS

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Mechanical properties and good biocompatibility of zirconium and some of its alloys made these materials suitable for biomedical applications. Although some *in vivo* studies have shown that zirconium and its alloys promote the osseointegration and that its citotoxicity is very low the use of this material has not been extended, and information about its performance is scarce.
The good *in vivo* performance of zirconium is mainly due to the presence of a protective oxide layer formed in air or in oxygenated electrolytes. This film diminishes the corrosion rate, minimizing the metal ion release to the biological media and promoting its osseointegration. Porosity control is a desired quality to enhance the facility of bone formation in contact with the implant. Although zirconium has a thermodynamic tendency to form an adherent ZrO2 surface film in air atmosphere, the films are very thin, with thicknesses between 2 and 5 nm. Porosity can be improved increasing the film thickness, having the additional advantage of enhancing its barrier effect. The oxide film can be thickened by means of anodizing, or thermal treatments.
Since the implant surface plays a key role in the living tissue response, the surface characterization of materials employed in orthopaedic surgery is a topic of concern. An extensive and systematic study of the influence of surface characteristics on the biocompatibility of zirconium *in vivo* and *in vitro* is required if this material is proposed as an alternative to titanium alloys in permanent implants. With this aim, this first part of the study is devoted to the electrochemical and surface characterization of zirconium surface film *in vitro*, in the "as received" material and after various surface modification processes as anodic oxidation or electrochemical deposition in comparison with commercially pure titanium. The characterization techniques include scanning electron microscopy with X-ray microanalysis, atomic force microscopy, X-ray photoelectron spectroscopy, polarization curves and electrochemical impedance spectroscopy. Finally, the bioactivity zirconium with different surface finishing are studied by means of apatite formation tests in a simulated body fluid solution (SBF).

BM10.

FIXATION OF METHYLENE BLUE TO BIOCOMPOSITES FILMS BASED ON CHITOSAN AND THEIR CHARACTERIZATION

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In this work we studied the formation of biocomposite films through attaching of chitosan (CS) on polypropylene (PP) modified with grafted acrylic acid (AAc) and their use in the fixation of methylene blue (MB) like a model substrate. Methylene blue, a molecule with anti-oxidizing and antibacterial activity, was attached to the film surface in order to analyze its incorporation performance at different superficial levels. Firstly, surface of PP film was modified with AAc using a photo-graft polymerization at room temperature, and benzophenone (BP) was used as radical initiator. Here, the effect of the reaction time was analyzed on the AAc grafting degree (G) during the reaction. Later, we analyze the effect of the AAc grafting degree (G) on the reaction of CS immobilization (I) and final properties of the biocomposite films attained. It was found that the surface modification of PP films with 16% of G shows the more suitable properties to develop the CS-immobilization reaction, wich consists in electrostatic bond onto the carboxyl groups of the grafted film (PP-g-AAc), obtaining also the biocomposite films (PP-g-AAc-CS). The maximum value of CS immobilization obtained at room temperature was a 8%. The MB dye was fixed to films modified using three techniques, where in a case the MB molecule was attached to PP-g-AAc by electrostatic interaction with carboxyl groups (A). On the other hand, the dye was fixed into the PP-g-AAc-CS film by diffusion (B), and finally, methylene blue was mixed with CS and then anchored to PP-g-AAc (C).
Key words: *chitosan, films, packaging, polypropylene*

BM11.

FABRICATION AND CHARACTERIZATION OF HAEMOCOMPATIBLE TiO_2 FILMS BY THERMAL AND ANODIC OXIDATION TECHNIQUES

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A protective titanium dioxide (TiO_2) film covers the surface of the titanium (Ti) and its alloys in ambient conditions. This natural oxide is 2 to 7 nm thick, and it is one of the main responsible for the success of titanium alloys as an implantable material. Thicker TiO_2 films can be produced on a titanium alloy, as the Ti-6Al-4V, by thermal or anodic oxidation. In this study, TiO_2 films on Ti-6Al-4V as substrate were produced by thermal and anodic oxidation techniques and compared. On one hand, TiO_2 films produced by thermal oxidation were growth in air atmosphere at 500 and 600ºC during different times of process: 0.5, 1, 2 and 24 hour. On the other hand, TiO_2 films produced by anodic oxidation technique were growth in a 1M H_2SO_4 solution at voltages from 10V to 100V, during 1 min. The surface characterization was performed by optical and scanning electron microscopy, and by X-ray diffraction (XRD) operated with glancing angle geometry with an angle of incidence of 1°. Films of different colours were obtained, with thickness ranged from 20 to 200 nm. Smooth and compact films were obtained at time processes below 1 hour by the thermal technique and at voltages below 50V by the anodic technique. Porous films were obtained at long times and voltages larger than 50V. Crystalline phases of TiO_2 (anatase and rutile) were obtained by thermal treatment and by the anodic treatment at high voltages. The films produced at low voltages are amorphous; these amorphous films could be transformed to crystalline by a thermal treatment with a slightly increase of the thickness and no change in the roughness. These smooth and crystalline films are suitable to biomedical applications and devices in contact with blood, were haemocompatibility are necessary.
Key words: *titanium oxide films, anodic oxidation, thermal oxidation, haemocompatibility, crystalline films.*

BM12.
NANOFIBROUS SCAFFOLDS CONTAINING CONDUCTIVE NANOPARTICLES
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Nanofibrous polymeric scaffolds have biomimetic features and can create an extracellular matrix architecture in a nanoscale way. Recognizing the importance of electrical and mechanical properties for several tissue reconstruction, conductive nanofibrous scaffolds are interesting candidates for tissue engineering and biotechnological applications. In this work we report the development of polyurethane-based nanocomposite scaffolds with conductive properties obtained by electrospinning. Electrospun polyurethane scaffolds containing single-walled carbon nanotubes (SWCNT) or polyaniline (PANI) 0.5, 1, 1.5 and 2% wt, were prepared. Medical-grade aliphatic polyurethane (Tecoflex 60D) was dissolved in dimethylformamide. The polyurethane solutions were mixed with suspended nanoparticle in tetrahydrofuran and the mixture sonicated until complete homogenization. The content and dispersion of nanoparticles played an important role in the electrospinnability of the solutions. The processing conditions were optimized in order to obtain uniform bead-free nanofibrous membranes. The residual solvent was completely removed under vacuum and the morphology of the prepared membranes was examined by SEM. An effect in the microstructure was found,: the mean diameter of the nanofibers decreased with the increase in the nanoparticle content. For 1.5% wt SWCNT a mean diameter of 464 nm was measured, while in the case of the same concentration of PANI a 430 nm in diameter was observed. In all cases, a unimodal diameter distribution was obtained. Conductivity measurements were performed by four-probe method, and by impedance measurements using a LCR meter. The scaffolds containing polyaniline nanotubes presented significant higher conductivity values. Moreover, both composites showed an increase in the conductivity, both a.c and d.c. conductivity, with an increase in the nanoparticles concentration. However, with both types of composites the nanoparticle content exhibited an upper limit, above which the conductivity decreased. Suspensions with high conductivity have a greater charge carrying capacity than suspensions with low conductivity. Thus, fiber jets of highly conductive solutions are subjected to a greater tensile force in the presence of an electric field, yielding more uniform fibers with smaller diameter. This effect was observed in the scaffold microstructure obtained from solutions containing PANI and SWCNT. Further studies on the nanoparticle dispersion-aggregation in the nanofiber and nanofiber roughness are under progress.
Key words: *nanofiber, scaffold, nanocomposite, conductivity.*

BM13.
DEVELOPMENT OF POLYURETHANE SCAFFOLDS FOR SOFT TISSUE-ENGINEERING APPLICATIONS
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The development of biomimetic highly-porous scaffolds is essential for successful tissue engineering. Segmented poly (ester urethane)s and poly(ester urethane urea)s have been infrequently used for the fabrication of electrospun nanofibrous tissues, which is surprising because these polymers represent a very large variety of materials with tailored properties. This study reports the preparation of new electrospun elastomeric polyurethane scaffolds. Two novel biodegradable segmented polyurethanes, synthesized from poly(ε-caprolactone) diol, 1,6-hexamethylene diisocyanate, and diester-diphenol or diurea-diol chain extenders, were used. The electrospinnability and the morphology of the electrospun SPU scaffolds were investigated and discussed. The electrospinning parameters such as solution properties (polymer concentration and solvent) and processing parameters (applied electric field, needle to collector distance and solution flow rate) were optimized to achieve smooth, uniform bead-free fibers mimicking the protein fibers of native extracellular matrix. The poly(ester urethane urea) was not possible to electrospin into uniform matrices employing *N,N*-dimethylacetamide (DMAc), and DMAc/acetone and *N,N*-dimethylformamide (DMF)/tetrahydrofuran (THF) solvent mixtures, whereas electrospun scaffolds from HPF solutions resulted in uniform bead-free-fiber matrices with a diameter average of 1.22 ± 0.42 μm. On the other side, the poly(ester urethane) could be processed from DMF/THF solutions, achieving fibers with a diameter average of 1.31 ± 0.82 μm, while electrospinning from 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) solutions resulted in matrices with a narrower diameter distribution (1.39 ± 0.56 μm). Thus, HFP is a better solvent than DMF/THF and DMAc/acetone solvent mixtures for electrospinning the present polyurethanes. The obtained elastomeric polyurethane scaffolds could be appropriate for soft tissue-engineering applications.
Key words: *scaffold, polyurethane, elastomer, soft-tissue engineering.*

BIOMATERIALS

BM14.

ABOUT THE TRANSITION TEMPERATURES MEASUREMENT IN NITINOL

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The use of nitinol (NiTi alloy) in biomedical applications is well established because this alloy has shape memory effect (SME) and good biocompatibility. The SME is due to a phase transition between two phases, austenite and martensite, characterized by four temperatures: Ms (martensite start), Mf (martensite finish), As (austenite start) and Af (austenite finish). The accuracy of these transition temperatures is very important in the design of an actuator to be used in biomedical applications, because phase transformation must occur close to body temperature. There are several methods reported to determine these temperatures: Differential Scanning Calorimeter (DSC), the most accepted one, Active Af, Resistivity Variation and Constant Load, but their accuracy and coherence are not clear yet. In this work the transition temperatures of NiTi wire under different heat treatment conditions were measured with three of these methods, that is, DSC, Active Af and Constant Load. The commercial wire (0,4 mm diameter) was solution heat treated at temperatures between 300 and 600ºC during half an hour, and rapidly quenched in water.

DSC and Active Af are ASTM standardized methods where the phase transition is determined without an applied load. Whereas DSC can determine the four temperatures, only As and Af temperatures can be obtained by the Active Af method. The Constant Load method developed in this work indicates the four temperatures under an applied load and a macroscopic displacement determine the transformations. The wire was mounted in flexion, submerged in a recipient with the thermostatized bath, and a small load was applied at its center. The temperature bath was varied between -60 to 60ºC at 2ºC/min, using a mix of alcohol with liquid air and boiling water to heat the bath over ambient temperature.

The results show that the transition temperatures obtained by the DSC method are not the same than the temperatures determined by the other two methods, which are similar. Also, the range between As-Af and Ms-Mf appeared to be higher than the DSC method. The Constant Load method is more effective for providing practical information about an actuator working with an applied load.

Key words: *nitinol, transition temperature, measurement methods, actuators*

BM15.

GELCASTING PROTOTYPING OF METAL POROUS PROSTHESES: PRELIMINARY TESTS

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The gelcasting prototyping, GP, is a new moulding process of prototypes of porous metal prostheses for total hip joint replacement. GP is based on formulation of metal slurries composed by 70-80% (by weight) of metal particles (up to 90 μm) of AISI 316 (surgical steel). These are dispersed in water solutions of natural proteins (up to 20% by weight of water), homogenised by mixing. The stabilized fluids are cast into ceramic shells and plastic mould, used to mould hip stems and acetabular components, respectively, and where the protein coagulation is induced by heating (80-90°C). The green pieces are composites of organic hydrogel matrix with anchored metal particles. The formed greens in plastic moulds are un-moulded, while the stems are preserved in partially opened ceramic shells. Both kinds of pieces are subject to thermal cycle, carried out under continuous hydrogen flow. This cycle is composed by: dehydration; pyrolisis of organic compounds; heating at 600°C h^{-1} up to sintering temperature (close to 1190 C), where stand by 2 hours, and cooling down at 600°C h^{-1} to room temperature.

The obtained pieces have porosities in the 43-60% range of their volume, which are mostly composed by interconnected porosity (78-98% of whole porosity). These micro-structures are able to absorb considerable amount of water solutions, such as antibiotics used for bone local infection treatments. The fluid up take could reach up to 4-9 and 15-20 cm^3 in acetabular and stem components, respectively. The pieces are more porous than in bulk, in 100-200 μm thick outer layers, which exhibit rough surfaces. Their biocompatibility and osseointegration ability were assessed before by *in vivo* tests. The materials have densities in the 3.5-5.0 g cm^{-3} range, contributing to reduce the effective weight of the prostheses.

The slurry formulation allows to control the porosity, density and surface finish in only one process, without the need of further steps. This task is impossible to do with conventional metal processing. Thus, the GP process is eligible candidate for costume made prostheses, built from diagnostic medical imaging and new designs of cementless orthopaedic devices. The GP costs are much more affordable than usual metal prototyping techniques, because the required moulds are machined from thermoplastic materials.

Key words: *metal prostheses, prostheses prototyping, prototype gelcasting, porous metal.*

BM16.
GELCASTING OF HYDROXYAPATITE-ZIRCONIA SCAFFOLDS

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The feasibility of manufacturing hydroxyapatite-zirconia, HA-Z, composite scaffolds devoted to bone reconstruction is analyzed. The use of these ceramics is the first step to form adequate interfaces between zirconia based ceramics, used in several dental and orthopaedic implants, with porous bone scaffolds, based on calcium phosphates with known osseo-conductivity. This goal leads to prepare two series of ZrO_2-yCaO (y = 8 or 16 molar % of Z) blends with hydroxyapatite, up to 50% in weight. These ceramics were dispersed in water solutions of egg albumin (up to 20% in wt.), at different ceramic contents. The employ of albumin had double roll: the air trapping, foaming of slurries, the slurry gelling. In addition, CaO promoted: the rapid increase of slurries viscosity, making them able to inject into moulds; the early protein coagulation (finished by further heating at 90 C), and the structural stabilization of zirconia. These slurries were moulded as simple porous plates and as light weight acetabular cups and hip spacers for hip prostheses. The un-moulded greens were slowly dried, and heated in order to pyrolize organics, until reach the sintering temperature (1350 C), where stand by 90 min, finishing with cooling down to room temperature in air. The obtained ceramic pieces have a multiphase structure (according to XRD analyses) composed mainly by calcium zirconates, ZA, product of HA reaction with Z, HA and β-tricalcium phosphate, product of partial HA des-composition, and known by its rapid absorption in organism. The formed ZA phase grows with Z contents, up to 60% of composite volume. These composites are porous in 50-70%, thus they have big water solution absorption ability, valuable aspect for application of scaffolds as drug realise systems. The mass transport, observed by SEM, is limited and restrained the bonding strength to magnitudes smaller than 5 MPa. This stress out the necessity of further increase of sintering temperature, even at expenses of more pronounced HA reaction with Z, and HA des-composition.
Key words: *zirconia Hydroxyapatite composites, gelcasting, biomaterial, bone scaffolds, ceramic prostheses*

BM17.
SYNTHESIS OF TiO_2 FILMS FROM SOL-GEL PROCESS: INFLUENCE OF THE SUPPORT AND THE SUPERFICIAL TREATMENT

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In this work sol-gel process to produce TiO_2 coatings on two different substrates, titanium and stainless steel, is used. The sol-gel process is an effective technique for the design of materials for various applications, which manages specific properties since early synthesis steps by controlling experimental parameters that allow the management of chemistry at the molecular level of the material. Thus, by varying the synthesis conditions characteristics such as pore size, morphology and particle size, crystal structure, synthesis mechanism, among others, can be controlled.
We analyze the influence of different surface treatments made to the substrate on the process of synthesis and deposition of the corresponding oxide. The other process parameters are kept constant.
These combinations substrate / coating have been previously studied from the viewpoint of the production of bioactive materials INOX/TiO_2 and Ti/TiO_2, specifically for implants with requirements of high mechanical resistance. They emphasize the mechanical properties, structural and biocompatibility of these materials, but it is not detected on literature studies of the mechanism for obtaining a layer of titania, which includes the influence of substrate used and its surface treatments.
The chemical precursor used in this work to produce the coatings is titanium butoxide (IV). This precursor was dissolved in ethanol in a 1:1 molar ratio, acidifying with acetic acid, with the aim of decreasing the speed of hydrolysis reaction, which is produced by contact with ambient moisture. Under these conditions the formation rate of the film is low enough to allow the observation of the different reaction steps using optical microscopy (OM).
Experiments were performed with the base substrates untreated, treated by mechanical abrasion and treated in strong acid medium.
All materials (supports, treated supports and coatings) were characterized by scanning electron microscopy (SEM), electron dispersion X-ray (EDS), X-ray diffraction (XRD) and optical microscopy (OM).
It was determined the influence of support material and various surface treatments on the synthesis process and on the characteristics of TiO_2 films obtained.
Key words: *biomaterials, titanium, TiO_2, sol-gel.*

BM18.
ALUMINA WITH GRADIENT OF PROPERTIES
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The technological advances occurred in the last decades have provided an improvement on the quality of living of the population to several areas of knowledge and consequently a guarantee of an increasing life expectation. The use of materials which can substitute bone parts has been reason of studies for many years. Thus, the materials must own chemical and mechanical properties similar to the ones in our organism. Such materials as: ceramic, plastic, metals or even the combination between them have been utilized as biomaterials. A line of ceramic materials that can be highlighted are the materials alumina based, due to their functions of biocompatibility and excellent mechanical properties. Samples with composition gradient were prepared, and for that $Al(OH)_3$ was added at quantities of 0, 5, 10 and 20% in mass. Afterwards, they were sensitized at 1550ºC with 240 minute isotherm in the air. Compression strength tests were taken in order to verify the influence of $Al(OH)_3$ on the densification of Al_2O_3 on account of the tension applied. The mechanical resistances to flexion were determined as four points as well. The compression strength tests showed that the higher the compression of $Al(OH)_3$ is the higher the apparent density of the sample becomes. On the other hand, the mechanical tests showed that the highest pressure supported was of approximately 20 MPa. It is concluded that the addition of $Al(OH)_3$ to ceramic mould influenced its properties, what can be studied later in order to develop a similar material.
Key words: *biomaterial, aluminum hydroxide*

BM19.
SYNTHESIS AND CHARACTERIZATION OF NEW HYDROGELS WITH pH-DEPENDENT SWELLING PROPERTIES. POSSIBLE APPLICATIONS AS BIOMATERIALS
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Hydrogels are polymeric materials that exhibit the ability to absorb huge volumes of water. In addition, the water absorption is reversible and dependent of the swelling conditions (pH, T, etc). As consequence of these properties, these kind of polymeric network have received attention as materials for biological, biomedical, pharmaceutical and agricultural applications. The knowledge of the swelling, the diffusion processes, the networks parameters that define its structure and the rheological properties in different conditions are very important since they will define their possible applications. In this work we present the synthesis and characterization of new hydrogels based in a new dendritic monomer with three acid groups (synthesized in our laboratory) with different amount of a crosslinking agent. Besides, thinking in useful application the studies of their properties were examined. The amount of fluid absorbed was evaluated for each hydrogel in water and at different pH. The swelling behavior was dependent of the amount of crosslinking agent and the pH of the fluid absorbed. More amount of crosslinking agent into hydrogel structures presented less equilibrium swelling ratio capacity ($\%SR_e$)in all swelling media. The major $\%SR_e$ was obtained in deionizated water. Besides, the hydrogels had different $\%SR_e$ at distinct pHs (from buffer solutions). The major swelling capacity was found at pH 7 and the minor at pH 3. At pH 7, the hydrogel is negatively charged and produces repulsion of charges that generates expansion of the network and more absorption of fluid. On the other hand, at pH 3 the hydrogel is totally protonated and the repulsion charge effect disappears, so the hydrogels are less swelled. The elastic moduli *G*' of the swelled matrices (at 25ºC in deionized) water were measured in a rotational rheometer. The strain sweeps and frequency sweeps tests were performed on each sample to determine the linear viscoelastic region profiles and viscoelastic performance in a range of frequencies. As expect, the material with more amount of crosslinking agent presented a critical shear stress (limit of linear response) some times larger than the other hydrogels. The frequency sweep test showed that the storage modulus are practically not influenced by the frequency of oscillation for the hydrogels in the range of frequency assayed (0.1-100 s^{-1}). The hydrogels developed in this study exhibit pH-dependent swelling and good viscoelastic properties suggesting that they have potential applications in various fields.
Key words: *hydrogels, polymeric networks, biomaterials*

BM20.

EXPRESSION OF INTEGRIN $\alpha_v\beta_3$ IN THE QUAIL EMBRYO CHORIOALLANTOIC MEMBRANE IN RESPONSE TO IONIC DISSOLUTION PRODUCTS OF BIOACTIVE GLASSES

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A number of *in-vitro* and *in-vivo* studies have highlighted the angiogenic potential of bioactive glasses (BGs), in particular the composition 45S5 Bioglass®. However, the cellular and molecular mechanisms by which BGs affects angiogenesis remain to be elucidated. The aim of the present study was to determine whether the ionic dissolution products of melt-derived BGs affect the expression of integrin $\alpha_v\beta_3$, a marker of angiogenic vascular tissue, employing the quail embryo chorioallantoic membrane (CAM) as the experimental model of angiogenesis. Fertilized eggs of Japanese quail (*Coturnix coturnix japonica*) were incubated *in-ovo* at 37ºC under ambient atmosphere, cracked at embryonic day 3 (E3) into 10-cm^2 wells of tissue culture polystyrene, and cultured further *ex-ovo* at 37ºC. The extract containing the dissolution products of BGs was prepared by soaking 1% w/v 45S5 BG particles (< 5 µm) of composition (in wt.%): 45% SiO_2, 24.5% Na_2O, 24.5% CaO and 6% P_2O_5 or 45S5 BG containing B_2O_3 (2 % wt) (labeled 45S5.2B) in Hank's balanced salt solution (HBSS, Gibco, pH 7.4) in an orbital shaker at 37ºC for 72 h. The elementary content of calcium (Ca), silicon (Si), boron (B), phosphorus (P) and sodium (Na) in the filtered extracts were determined by ICP analysis. At E7, 0.5 mL prewarmed BG-conditioned HBSS solutions were applied dropwise to the surface of each CAM. HBSS without added BG-ionic dissolution products served as control. The embryos were incubated further at 37ºC for 24 h. An enzyme-linked immunoadsorbent assay (ELISA) was performed to measure the endogenous levels of $\alpha_v\beta_3$ in the CAM after treatment. Briefly, CAMs were homogenized in RIPA buffer, and 2-4 µg of total protein as determined by the Bradford protein assay was coated in triplicate in each well of a 96-well plastic dish. The plates were dried at 37ºC, blocked in PBS/5%BSA, incubated with an anti-$\alpha_v\beta_3$ primary monoclonal antibody (LM609, 1µg/mL), washed with ELISA wash buffer, incubated with horseradish peroxidase-conjugated anti-mouse secondary antibody, and developed in *o*-phenylenediamide. Plates were read in an ELISA scanner at 492 nm. The $\alpha_v\beta_3$ levels remained unchanged in CAMs exposed to a single application of control solution (HBSS) and /or BG-conditioned HBSS solutions for 24 h. The $\alpha_v\beta_3$ levels were not significantly increased above the baseline level detectable at developmental embryonic day 8 (E8) in untreated CAMs. The ionic dissolution products from BGs 45S5 and/or 45S5.2B used in the present work did not produce an angiogenic response, as determined by ELISA assay for $\alpha_v\beta_3$ expression.

Key words: *Bioactive glass, angiogenesis*

DRUG DELIVERY

DD1.

DEVELOPMENT OF AN INJECTABLE *IN SITU* MODIFIED RELEASE SYSTEM FOR VETERINARY USE: POLOXAMER AND POLYELECTROLYTES PLATFORMS OF RELEASE

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The aim of the present work it is to explore the potential of combining Poloxamer 407 (PO) and polyelectrolytes (PE) as an *in situ* injectable release system for veterinary use, using Progesterone (Prg) as model drug, and evaluating the release and erosion from the developed formulations. PO, Carragenina (CA), Alginic Acid (AA), Sodium Carboxy methyl cellulose (CM) and Prg were used to prepare the evaluated formulations. To obtain *in vitro* release profiles; the membraneless model was used at 38 ± 0.05ºC, with 10 mL of physiological solution as release medium. At predetermined intervals of time, the release medium was completely replaced by new one, which was supported at 38 ± 0.05ºC. It was determining the weight of the formulations to calculate the proportion of dissolved gel. Prg's release rate is influenced by different ways, according to the PE incorporated in the release platform of PO-Prg. For example, when the gear incorporates CM it increases about a 20%. Whereas the aggregate of AA practically does not influence (3%) and CA's presence decreases the release rate in about 10%. It is noticed that in all the evaluated systems the erosion increases in with the incorporation of the macromolecules in the following order CM >AA >CA. These results are coincidental with the data of release obtained. PE's incorporation influences significantly in the release and erosion of PO-Prg systems, allowing to select formulations with *in vitro* optimal properties for the development of an injectable *in situ* delivery system for veterinary medicine use.

Key words: *modified release, poloxamer 407, polyelectrolites*

DD2.

ESTRADIOL PERMEATION STUDIES IN CHITOSAN MEMBRANES. EFFECTS OF CROSS-LINKING TIME

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Chitosan (CHT) is an abundant, low cost, biodegradable, biocompatible and non-toxic polysaccharide with good membrane and gel forming properties. Pharmaceutical formulations containing estradiol (E2) are used by women for the treatment of menopausal symptoms and menstrual disorders. This female hormone is also a contaminant present in wastewater effluent. The study deals with the analysis of the interaction between E2 and CHT, in particular the influence of cross-linking density of CHT membranes on swelling, sodium content and E2 permeation. CHT powder was characterized for its degree of deacetylation (77%) and viscosimetric molecular weight (600KDa). CHT membranes were obtained by a casting/solvent evaporation method and cross-linking with sodium tripolyphosphate (TPP). The cross-linking conditions were: an aqueous solution of TPP 5% w/v and cross-linking time: 5 to 55 min. Sodium content increased with increasing cross-linking time, in a logarithmic manner, from 11.61 to 40.43 μg mg^{-1} of membrane. Membranes exhibited an increment in equilibrium water content (0.44 to 0.647 g g^{-1}) and flux of E2 (2.458 to 12.743 μg cm^{-2} h^{-1}) with the increase in cross-linking duration from 15 to 55 min. One explanation for these results could be the altered type of linkage between CHT and TPP (because of the prolongation of the cross-linking reaction), or the existence of a significant correlation between the hydrophilicity of the membrane and the low water solubility of E2. Hydrophobic compound are less solvated and can enter more easily into the membrane pores. The exception was the membrane with the lowest cross-linking time which showed the higher equilibrium water content and a higher flux of E2 compared with the others membranes, this could be attributed to improper cross-linking. This knowledge could have a great potential application in the recycling of wastewater effluents using CHT membranes for the removal of E2 (a currently contaminant) or in the pharmaceutical industry for the development of transdermal delivery systems.

Key words: *chitosan membrane, cross-linking, estradiol, water content, drug permeation.*

DD3.

MEGAMERS (MG): A NEW NANO DRUG DELIVERY SYSTEM?

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Polypeptide or oligopeptide drugs have significant therapeutic potential in the clinics, but their poor oral bioavailability, mainly due to human body's natural defence and digestive mechanisms, requires these agents to be administered primarily by parenteral routes. Their short plasma half-lives and the poor results obtained upon repeated parenteral injections have led to the search for novel delivery devices that allow for friendly routes of administration, for instance the oral route.

In order to allow the oral administration of polypeptides, the maintenance of their chemical structure along the gastrointestinal transit, mucoadhesion and increased possibilities of para-celular transport across the enterocytes must be achieved. To that aim, we propose the polypeptides to form complexes with structure-controlled MG -core-shell nanoparticles formed by dendrimers-.

The synthesis of the MG, saturated core-shell (tecto) dendrimers, involved the combination of a limited amount of an amine-terminated dendrimeric core reagent (PAMAM dendrimer of generation (G) 5, with an excess of a carboxylic acid terminated dendrimeric shell reagent G 2,5, whit a subsequent covalent bond formation. The resultant product was purified by size exclusion chromatography (SEC) and lyophilized as a white rubbery solid. Formation of MG was determined by: a) SDS-PAGE (polyAcrylamide gel electrophoresis) b) HPLC (high performance liquid chromatography) c) SEC d) size and Z potential by NanoZ sizer and e) thermogravimetric analysis (TGA).

MG showed to be structurally stable, monodisperse, with a molecular size distribution of 8 ± 1.5 nm and a MW of 95000 Da. Finally, FITC labelled MG showed to cross the epithelial barrier when incubated with Caco-2 cells, in the absence of cytotoxicity, measured as mitochondrial succinate dehydrogenase activity employing a tetrazolium salt (MTT) and as lactate dehydrogenase (LDH) leakage.

MG demonstrated to be a promising device to deliver intact molecules of polypeptides towards the epithelial barrier in an oral administration.

Key words: *nanoparticles, megamer, dendrimer, polypeptides, oral delivery.*

DD4.
CHARACTERIZATION OF MICROSPHERES FORMED IN SITU FOR DRUG-CONTROLLED RELEASE
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Biocompatible and biodegradable poly(lactic-co-glycolic) acid (PLGA) are widely used for drug delivery systems. Microspheres formed in situ are a novel and practical dosage form of the PLGA-based controlled release devices. They are prepared by dissolving the polymer and the drug in a solvent-system (phase 1) and then emulsified within a second solvent-system (phase 2). When the emulsion (pre-microspheres or microglobules) comes in contact with water or physiological fluids, organic solvents are diluted and the polymer precipitates entrapping the drug and forming spherical microparticles. In order to develop a drug-eluting platform based upon this concept, we perform an experimental study to choose the most appropriate solvent-system that yields in situ formed microspheres. PLGA formulations comprising 2-Pyrrolidone (2P), Glycerol Formal (GF) and mixtures of GF and Triacetin (GFT) were tested as solvents forming the phase 1, while Miglyol 812 (M) was used as the oily phase 2. PLGA 50:50 (Resomer) solutions at 20 and 40% w/w in phase 1 were cast into phase 2 and vigorous stirred to form pre-microspheres. The resulting emulsion was cast into an aqueous buffered solution (pH=7.4) to form the microspheres. After analysing size distribution (mean ± SD), shape and sphere surface by optic microscopy and scanning electron microscopy (SEM) the following conclusions arisen up to the present. The more hydrophobic phase 1 systems (GTF) with 20% of PLGA yielded the highest mean size (116.1 ± 63.5 and 103.5 ± 48.2 µm) with values ranged from 34.5 to 375 µm. The systems with 2P rendered a lower size (88.5 and 52.8 µm for 20 and 40%, respectively) but a great dispersion (49.3 and 74.8% RSD). The systems with GF gave a size distribution between 14.6 and 156.6 µm with means of 61.5 ± 27.4 (44.6% RSD) and 39.6 ± 13.2 µm (33.3% RSD) for 20 and 40% of PLGA, respectively. For all solvent systems, the shape of the microspheres of 40% PLGA was more spherical than 20% PLGA, but GF conserved this shape at 20% more than others. The sphere surface was more porous for systems using GF than 2P. The GTF were not characterized. The microscopic characterization showed that the in situ formed microparticles are spherical and non-aggregated particles characterized by a Gaussian size distribution. The best solvent was GF because the microspheres exhibited appropriate sizes and a more uniform distribution, at both high and low PLGA concentrations. Its higher surface porosity may increase the diffusion phenomena and erosion of PLGA. These characteristics make it a promising system for the controlled release of drugs.
Key words: *PLGA, controlled release, in situ formed microsphere, size distribution*

DD5.
ALENDRONATE CONTROLLED RELEASE USING ACRYLIC MEMBRANES
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Alendronate is a nitrogen-containing bisphosphonate that not only act on the osteoclasts, inhibiting bone resorption, but also have direct effects on osteoblasts, osteocytes, and macrophages, thus regulating the function of different skeletal cells. In general, bisphosphonates are synthetic analogues of pyrophosphates that are attached to the hydroxyapatite in the bone. In our country alendronate is indicated for the treatment of osteoporosis associated with both menopause and the use of corticosteroids.
The development polymeric membranes able to support alendronate as a drug delivery system could have the advantage to bypass gastrointestinal absorption, to eliminate the first step hepatic effect and to reduce the incidence of secondary effects due to the decrease in the used doses. That, our group has developed biocompatible membranes of acrylic polymer for alendronate delivery system. The commercial acrylic polymer used to manufacture these membranes was Duro Tak 87-2852 (Nationals Adhesives Company). Membranes containing 1% sodium tri hydrate alendronate (Elea Laboratories,Argentina) were prepared into 5 cm diameter petri dishes pre-covered with a aluminum foil. Alendronate dispersed in PEG400 was added to the acrylic polymer and allowed to evaporate the organic solvents into a hood for 48 hours. Total evaporation of the solvents was controlled to constant weight.
Sodium alendronate membranes (250 µ thick) were tested under the conditions of release described in the USP 2007, using a K_2PO_4H - KPO_4H_2 buffer, pH = 7.4, 35.0 ± 1°C and rotational blades speed of 50 rpm. Samples were extracted at different periods of times.
The quantification of the released alendronate was performed by a method based on the molar ratio of mononuclears complexes applied in this case the ratio between the Fe (III) and alendronate (Jovan Nedeljkovic *et al*., J. Pharm. And Biomed. Anal. 28, 1215 1220 (2002))
The results of dissolution tests show that the release occurs in a controlled manner during the time (about 50 hs). According to our initial studies, it seem that this type of acrylic biocompatible membranes can be used as carriers of alendronate. Besides, if the diffusion tests are expected, it will be possible that these membranes can be used as a transdermal devices.
Key words: *release of sodium alendronate, acrylic membranes of alendronate*