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1. EDITORIAL: REGENERATIVE MEDICINE – A CHALLENGING DOMAIN OF RESEARCH IN XXIST CENTURY

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Regenerative medicine stands at the intersection of a variety of rapidly developing scientific disciplines: stem cell biology, immunology, tissue engineering, molecular biology, biomaterials, transplantation biology, and clinical research. Patients suffering from diseased and injured organs may be treated with transplanted organs. When organs or tissues are irreversibly injured, these could be replaced by artificial systems or by a similar organ from a donor. There is a severe shortage of donor organs which is worsening yearly due to the aging population. In addition to providing hope for the ageing and diseased, tissue engineering is already helping the large population of young people who participate in sports, many of whom suffer injuries. The development of some methods of tissue engineering which allow the replacement of organs through ex vivo culture of autologus cells, could represent a valid substitute for organ transplant, thus peventing the risks of transmitting some diseases from donors, a common problem in organ transplant.

Tissue engineering using cells, especialy stem cells, allows exploiting two important qualities of these cells: high proliferation capacity and multipotentiality of these imature cells. The new field of regenerative medicine is one of the most exciting fields in medicine and brings together for the first time the scientific disciplines of biology, materials science and biomedical engineering directed towards long-term repair and replacement of failing human tissues and organs. Living cells are harvested, grown in the laboratory, often on an appropriate scaffold, and stimulated to form specific tissues that mimic the complex structures and physiological behaviour of natural tissues. Ultimately, "spare parts" developed in the laboratory can then be put into a patient, either by injection of cells or by implantation of intact tissue or an entire organ. The stem cell field is advancing rapidly, opening new options for therapy in tissue engineering.

The goal of regenerative medicine is to repair organ pathologies such as those acquired congenitally or by cancer, trauma, infection, or inflammation. It is based upon the foundations of cell transplantation and materials science. The interest in modern biological technologies has dramatically increased since it is feasible to isolate living, healthy cells from the body, expand them under cell culture conditions, combine them with biocompatible carrier materials and re-transplant them into patients. Therefore, tissue engineering gives the opportunity to generate living substitutes for tissues and organs, which may overcome the drawbacks of classical tissue reconstruction: lacking quality and quantity of autologous grafts, immunogenicity of allogenic grafts and loosening of alloplastic implants. Due to the prerequisite for tissue engineering to ensure a sufficient number of

tissue specific cells without donor site morbidity, much attention has been drawn to multipotential progenitor cells such as embryonic stem cells, periosteal cells and mesenchymal stem cells [19]. According to Hipp J. and Atala A. [6], tissue can be engineered 1) *in vivo*- by stimulating the body's own regeneration response with the appropriate biomaterial, or 2) *ex vivo*- cells can be expanded in culture, attached to a scaffold and then re-implanted into the host. Cells may be heterologous (different species), allogeneic (same species, different individual), or autologous (same individual). Autologous cells are preferred because they will not evoke an immunologic response and thus the deleterious side effects of immunosuppressive agents can be avoided. While autologous cells are recognized as the ideal transplantation resource, many patients with end-stage organ disease are unable to yield sufficient cells for expansion and transplantation. Furthermore, some primary autologous human cells cannot be expanded from particular organs (*i.e*. pancreas, liver).

Stem cells are envisioned as being an alternate source of cells from which the desired tissue can be derived. Human embryonic stem cells (HESC) can be derived from discarded non transferred embryos and have the advantage of being pluripotent (the ability to differentiate into all tissues of the embryo) and able to self-renew indefinitely. However, their clinical application is limited because they represent an allogeneic resource and thus their use would require high dose immunosuppressive therapy.

The hope that many diseases can someday be treated with stem cell therapy is inspired by the historical success of bone marrow transplants in increasing the survival of patients with leukemia and other cancers, inherited blood disorders, and diseases of the immune system [25]. Nearly 40 years ago, the cell type responsible for those successes was identified as the hematopoietic stem cell [26]. The ability of hematopoietic stem cells (HSCs) to self-renew continuously in the marrow and to differentiate into the full complement of cell types found in blood qualifies them as the premier adult stem cells. HSCs are among the few stem cells to be isolated in adult humans. They reside in the bone marrow and under some conditions migrate to other tissues through the blood. HSCs are also normally found in the fetal liver and spleen and in umbilical cord and placenta blood. There is a growing body of evidence that HSCs are plastic—that, at least under some circumstances, they are able to participate in the generation of tissues other than those of the blood system. A few studies have shown that HSCs can give rise to liver cells [11, 24]. It was unexpected that a component of blood could cross over a developmental separation to form a tissue type that ordinarily has a completely different embryonic origin [11]. The findings noted above and other reports of cardiac and muscle tissue formation after bone marrow transplantation in mice [17] and of the development of neuron-like cells from bone marrow [2, 6] have raised expectations that HSCs will eventually be shown to be able to give rise to multiple cell types from all three germ layers. One study has, in fact, demonstrated that a single HSC transplanted into an irradiated mouse generated not only blood components (from the mesoderm layer of the embryo), but also epithelial cells in the lungs, gut, (endoderm layer) and skin (ectoderm layer) [10]. There is also evidence that transplants derived from umbilical cord blood are less likely to provoke graft versus host disease, possibly because the cells in cord blood are immature and less reactive immunologically [12]. The quantity of HSCs present in cord blood and its attached placenta is small, and transplants from cord blood take longer to graft, but for children, whose smaller bodies require fewer HSCs, cord blood transplants are valuable, especially when there is no related sibling to donate HSCs [4]. Banks of frozen umbilical cord and placenta blood (drawn out of the umbilical vein of the cord) are an important source of HSCs because the histocompatibility markers on the cells in these tissues can be identified and catalogued in advance of the need for a transplant. If HSCs are truly multipotent, their potential for life-saving regenerative therapies may be considerably expanded in the future.

Human mesenchymal stem cells (MSCs) - also known as stromal cells - are also used for cell therapies in different conditions associated with organs and tissues disorders. Multipotent mesenchymal stromal cells are endowed with multilineage differentiative potential and immunomodulatory properties. It is still a matter of debate whether donor MSCs have sustained engraftment potential in host bone marrow after allogeneic hematopoietic stem cell transplantation [18]. There are multiple sources of MSCs, some of them common to HSCs sources: bone marrow, peripheral blood adipose tissue, umbilical cord and umbilical cord blood, etc. They are known to differentiate into at least three cell lineages: osteocyte, adipocyte and chondrocyte, but are likely to be able to transdifferentiate and dedifferentiate into much more cell types, required for cell based

therapies. Their potential is still under investigation and the possibilities for differentiation into different cell types seem to be limitless. Even if the origin and the functions of MSCs are still unclear, their presence in the adult peripheral blood might relate to adult stem cell biology, such as the mechanism of systemic migration of bone marrow-derived multi-potent stromal cells and the existence of a common hematopoietic-mesenchymal precursors.

Specific therapeutic applications of tissue engineering research include, but are not limited to:

- Novel cell delivery models and approaches, including delivery of cells in scaffolds to promote healing for repair, replacement or regeneration of tissues;
- Development of scaffolds with appropriate characteristics to promote cell and tissue survival and integration;
- Development of novel animal and culture models for regenerative medicine applications, including innovative models of acute and chronic injury, aging models, organ cultures and co-culture systems;
- Molecular and biochemical basis of vascularization and angiogenesis in native and exogenously transplanted tissues and organs;
- Approaches to minimize cell death and promote cell survival and differentiation in transplants;
- Application of tissue-engineered biomaterials as conduits or shunts in tissue regeneration;
- Development of important new insights into "normal" structure, function and/or development of tissue and organ systems of interest;
- Development of effective new strategies for improving healing, repair, biological replacement or regeneration of tissue and organ systems of interest;
- Ethical, legal, social, cultural and economic consequences of regenerative medicine based on tissue engineering strategies.

There are several teams involved in the study of stem cells and their therapeutic potential. Their reports were published in acknowledged speciality journals (Nature, Science, PNAS, Blood, Stem Cells, NEJM, etc.) and are still the most challenging domain in medical research all over the world.

Cell therapy as base of regenerative medicine can address a number of human conditions from chronic to life threatening: organ transplant, Parkinson's disease, Alzheimer's, arthritis, osteoporosis, emphysema (smoker's lung), liver cirrhosis, diabetes, heart disease, trauma, sports injuries:

- **Heart diseases:** Minguell JJ. and co [15] revealed the benefits of regenerative medicine in heart diseases. Most attempts for cardiomyoplasty have considered the bone marrow as the source of the repair stem cell(s), assuming that the hematopoietic stem cell can be a choice. Since 1995 it has been known that under *in vitro* conditions, MSCs differentiate into cells exhibiting features of cardiomyocytes. Freyman T. [3] proved that intracoronary and endocardial injection of MSCs post- myocardial infarction resulted in increased engraftment within infarcted tissue when compared with intravenous infusion. Nagaya N and co [16] showed that MSC transplantation improved cardiac function in a rat model of dilated cardiomyopathy, possibly through induction of myogenesis and angiogenesis, as well as by inhibition of myocardial fibrosis.
- **Bone and cartilage disorders:** In the ortopedic field it was demonstrated the posibility of autologus human condrocytes or mezenchymal stem cells transplant in order to reconstruct the injured cartilage, as a result of trauma or some pathologic entities, and to enable the new bone tissue formation in treatment of skeletal anomalies, trauma and bone tumors. There is a conceptual change regarding hard tissues repair, in the way that there is no need to find materials that could replace the destroyed part of the bone, but we need to find materials that could help the organism to the process of auto-repair of the damaged tissues, such as bioactive materials of the third generation [7].
- **Diabetes mellitus:** Beta-cell replacement therapy via islet transplantation has received renewed interest due to the recent improved success. In order to make such a therapy available, new sources of insulin-producing cells must be readily available. The most promising sources are stem cells, with efforts of deriving new beta-cells from both embryonic and adult stem cells [21]. Under strong induction of pancreas regeneration, pancreatic duct cells dedifferentiate to grow, and re-differentiate toward other cell types including islet cells [23].

- **Neurodegenerative diseases:** New cell therapies will generate different types of neural cells for the treatment of degenerative diseases such as Alzheimer's and Parkinson's. It would be possible that spinal cord injury patients may regain full function of their body. Even some genetic disorders could be cured, not just treated [27].
- **Kidney diseases:** Stem cells offer an exciting potential for kidney regeneration. Lin F. [13] revealed evidences to support the conclusion that intra-renal cells, including surviving tubular epithelial cells and potential renal stem/progenitor cells, are the main source for renal regeneration. Future research will be focussed in selecting the type of stem cells, frequency and route of administration of the cells will be fundamental in successful cell replacement therapy in acute renal failure. Recent studies indicate the possibility of collecting autologous bladder cells from human patients, expanding them in culture, and returning them to the human donor in sufficient quantities for reconstructive purposes.
- **Muscle diseases:** According to Bach AD [1] there are two principle strategies for the replacement of impaired muscle tissues: (1) the application of isolated and differentiated cells (in vivo tissue engineering), using a transport matrix for the cell delivery; (2) use of in vitro-designed and pre-fabricated tissue equivalents (in vitro tissue engineering). Duchenne muscular dystrophy can be treated using bone marrow-derived human MSCs genetically modified with the full-length Dys-coding sequence [5].
- **Transplant:** MSCs can be used in novel therapeutic strategies designed to improve engraftment or to suppress graft-versus-host disease in bone marrow transplantation, due to the fact that MSCs can inhibit NK-cell proliferation [22]
- **Cancer:** Both human stem cells and mature stromal cells can play an important role in the development and growth of human malignancies. In contrast to these tumour-promoting properties, human MSCs home to sites of tumour-genesis and potently inhibit tumour growth. MSCs possess intrinsic antineoplastic properties and might be of particular utility for treating those human malignancies characterized by dysregulated Akt, as Kaposi's sarcoma [9].

The research on cell therapies in Romania: the enormous potential of stem cell research, both in novel therapeutics and in understanding the basic biology of malignancy, has driven some Romanian research units to initiate basic and clinical research in this field and to provide collaborative interactions with other scientists. The most advanced are the following:

- **The University of Medicine and Pharmacy Victor Babes Timisoara** (www.umft.ro) has as strategy in research to become an excellence centre in stem cell therapies till 2007. It is the leading Regional Centre specialized in stem cell therapies, with an existing Stem Cell Cluster of 9 departments (immunology and physiology, histology, biochemistry, biophysics, haematology, cardiology, paediatrics, orthopaedics, experimental microsurgery), organized around the Immune-physiology and Biotechnologies Centre, collaborating on a range of research projects involving stem cells of adult origin. The University has well equipped facilities for basic research, as well as for experimental studies and clinical trials and well-trained researchers involved in the field. It is the first university in Romania who began a clinical trial using stem cells (autologous transplantation of AC133+ bone marrow-derived stem cells in patients with acute and recent myocardial infarction), and participates to a FP6 integrated project in the field of stem cell research (GENOSTEM, LSHB 1.1-503161). The university is already partner in two European projects regarding Quality Management Systems, financed by EU Public Health Programme, Luxemburg (EU-QMS-BLOODNET and European Quality System for Tissue Banking - no 2003209). The mobility within EU is sustained by bilateral agreements with recognized EU universities (Frankfurt, Heidelberg, Montpellier), by Leonardo da Vinci Mobilities Programme, and by the training programme developed by GENOSTEM.
- **The University of Medicine and Pharmacy Carol Davila Bucuresti** (www.univermed-cdgm.ro) is specialized in the in vitro study of embryonic and human adult stem cell, with strong collaborative links with Karolinska Institute in this field and just won a Romanian LSH-SSA within the specific call for ACC/2004 in rheumatic disease study.
- **The Institute Nicolae Simionescu Bucharest** (www.icbp.ro) is an Excellence Center of Romanian Academy, specialized in the in vitro study of stem cells and its applications in cardio-vascular diseases, mainly in endothelial cells dysfunctions. The Institute has many national grants

in this field, and participates to FP5 as well as FP6 projects and is also recognized as EU Excellence Center. The Institute just won the other Romanian LSH-SSA within the specific call for ACC/2004 - SERA. The fundamental concept of SERA is to act as a support for reinforcement of our team and our country research capacity. The work is financially supported by the European Community, in the framework of "Integrating and Strengthening the European Research Area" – Specific Support Action. The activities are targeted to the Thematic Priority 1 of European Community FP6.

- The team of the **Clinical Institute Fundeni** (www.icfundeni.ro) under the lead of Prof. Irinel Popescu has many accomplishments and national premiers in surgical techniques. The most important of these is the introduction of the liver transplant in Romania, which is a very complex and laborious surgical procedure. Another accomplishment at the Clinical Institute Fundeni was in November 2005 when the first stem cell transplant for a liver disease has been performed. The stem cells have been isolated and prepared at the Cambridge University in the United Kingdom. This intervention is the 6^{th} out of 25 within a study performed by the Hammersmith Hospital, London and the Cambridge University.

The current state of the art for stem cell research seeks to: (1) explore adult stem cells in non-homologous settings, such as the use of HSCs in heart repair; (2) exploit further sources of stem cells, notably embryonic stem cells, for the treatment of paediatric, heart, pancreatic, liver and brain impairments; (3) use stem cell lines as tools in drug discovery and development; (4) increase the understanding and treatment of cancer by further studies of endogenous adult stem cells; (5) generate embryonic stem cells with the same nuclear genetic material to that of the patient using therapeutic cloning techniques, to avoid the potential rejection of cell therapies. Another ambitious goal for the field involves the use of endogenous stem cells, naturally resident in tissues of the human body, to direct the repair of damaged or diseased tissues. In all of the above examples, timescales are unknown and merely indicative [20].

In this regard, regenerative medicine may become a successful strategy by providing a dynamic, interactive, and individualized therapeutic approach that responds to the pathophysiological condition of the patient. Humes H.D. [8] revealed the fact that most common disease processes are not due to the deficiency of a single protein but develop due to alterations in the complex interactions of a variety of cell components. Cells may provide innovative methods for drug delivery of biologics, immunotherapy, and tissue regenerative or replacement engineering. The translation of this discipline to medical practice has tremendous potential, but in many applications technological issues need to be overcome. Since many cell-based indications are already being evaluated in the clinic, the field appears to be on the threshold of a number of successes. Collaborations between biotechnology and pharmaceutical companies with academic institutes and research centres will help overcome the technological challenges associated with the development of the stem cell-based therapies. For pharmaceutical companies, stem cells contribute to drug discovery through their application in finding novel drug targets and through the development of new technology platforms. Therefore, pharmaceutical companies will increasingly explore the different methods in which stem cells can be used in the drug discovery phase to accelerate the discovery of novel drug molecules. In time, regenerative medicine will also provide a cost-effective, long-term solution to many age-related and lethal conditions.

2. ROMANIAN SCIENTIFIC PROGRESS IN INTERNATIONAL STRESS RESEARCH AND STRESS - AGING MEDICINE

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ABSTRACT
The aim of this paper is to reveal the Romanian scientific progress in stress research and stress-aging medicine made by the Department of Stress Research and Prophylaxis, headed by Sorin Riga MD, PhD and Dan Riga, MD, PhD, at the "Al Obregia" Clinical Hospital of Psychiatry, Bucharest. Our synthesis is justified because the international publications and presentations of Drs Riga's team are

insufficiently well-known in Romania, compared to their significance and impact in bio-medicine and therapy.
In the background section we describe the stress globalization phenomenon, which is a major concern of the United Nations specialized structures: WHO, ILO, UNESCO. Advances in stress medicine and research reveal the quick progress and main directions in this field. Moreover, the paper includes the long-term contribution (1980-2005) made by the Academy of Medical Sciences and the University of Medicine from Cluj-Napoca, Romania.
In addition we characterized the new Romanian concepts and excellence in stress-aging research, bio-medicine, and therapeutics, ellaborated by Drs Riga's team, from 1972 until nowadays. Their concepts and research work in the field of neuroscience and anti-stress and anti-aging medicine are included in the diagnosis-therapy couple, applied at neuro-psycho-biologic level:

- anti-adaptive, anti-homeostatic and etio-pathogenic concept: distress ↔ impairment ↔ senescence ↔ disease; and
- homeostatic and orthomolecular therapy: anti-stress, anti-impairment and anti-aging.

Finally, we emphasized the international recognition of Romanian excellence in science and medicine owed to Drs Riga's department. Moreover, due to the medico-social actuality and important impact of stress and senescence, we underline the need to develop this department and its researches.

Key words: stress-aging medicine and therapy; Romanian scientific researches and progress; Department of Stress Research and Prophylaxis, "Al Obregia" Clinical Hospital of Psychiatry Bucharest.

3. CHANGES OF SOME OXIDATIVE STRESS MARKERS AND ANTIOXIDANT STATUS AFTER THE ADMINISTRATION OF VITAMIN E AND RESPECTIVELY SOY ISOFLAVONE IN FEMALE RATS WITH EXPERIMENTALLY INDUCED MENOPAUSE

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ABSTRACT
The aim of our study was to determine the effect of vitamin E and respectively soy isoflavone on serum levels of reactive oxygen species, antioxidants and nitric oxide in female rats with experimentally induced menopause. We found that an increased serum level of reactive oxygen species and decreased levels of antioxidants and nitric oxide accompanies experimentally induced menopause in rats. Soy isoflavone reduces significantly the level of free radicals in these rats. Vitamin E has significantly higher antioxidant effect than has soy isoflavone. Both vitamin E and soy isoflavone has antioxidant protective effects in ovariectomized rats, but the effect of vitamin E is stronger than that of soy isoflavone.

Key words: oxidative stress, menopause, isoflavone, vitamin E.

4. MICROFRACTURES IN FULL THICKNES CARTILAGE DEFECTS OF THE KNEE

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ABSTRACT
Cartilage lesions in the knee are still awaiting the optimal treatment method. Several techniques, starting with the old Pridie drilling and ending with themodern and expensive autologus chondrocite transplant (ACT) are not bringing the optimal results. Based on the stem cell potentials, the microfracture technique brings them into the defect region realising a compromise between the acceptable functional result and low costs especially in the light of the new studies which are establishing no difference between ACT and microfracture.

Key words: cartilage lesion, microfracture, autologus cartilage transplant

5. ABSTRACTS PRESENTED AT THE WORKSHOP: NEW STRUCTURES AND BIOMATERIALS FOR LIFE AND HEALTH. TRENDS AND PRIORITIES IN THE EUROPEAN RESEARCH AREA

ORGANIZERS:
Victor Babes University of Medicine and Pharmacy Timişoara
National R&D Programme New Materials, Micro and Nanotechnologies – MATNANTECH
Politechnics University Bucharest
April 14, 2006

1. ANGIOGENIC GENE THERAPY IN PATIENTS WITH SEVERE LOWER LIMB ISCHAEMIA

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ABSTRACT
The ongoing study focuses on the application of a therapeutical strategy in patients with chronic severe lower limbs ischaemia by means of plasmid vector encoding VEGF165 gene. The methodology for angiogenic gene therapy administration has been improved. A 1-year follow-up study in patients who underwent therapeutic angiogenesis will clinically assess its efficiency as well as the safety profile.
As part of this study, it has been verified the hypothesis that proposes that injection of a genetic material able to induce secretion of the angiogenic growth factors (VEGF165) into the critically ischemic lower limb will promote neovascularization with more efficient blood perfusion of the affected limb. This result is clinically demonstrated by disappearance of rest pain, healing of ischaemic ulcers and avoidance of amputation. The primary end-point has been the complete relief of rest pain and/or healing of ischaemic ulcers. Secondary outcomes have been the prolongation of walking time on the rolling carpet assessed by Gardner protocol, prevention of limb amputation, angiographic demonstration of newly visible collateral vessels as well as the safety of treatment (pathological angiogenesis will be evaluated: proliferative retinopathy, tumour growth, progression of atherosclerosis).
In the first stage, the vector has been designed to encode the gene of interest. Its purified form has been synthesized in sufficient quantity. VEGF165 mRNA has been isolated from human blood and used in complementary DNA synthesis by means of RT-PCR technique using random hexamer primers. VEGF gene amplification has been performed by PCR with specific oligonucleotide primers for the cDNA template. VEGF gene insert has been ligated into the plasmid mammalian expression vector – pCMV-Script Vector (Stratagene), and further cloned into E. coli strains.
E. coli colonies which incorporated the plasmid encoding VEGF have been isolated. The plasmid has been purified by affinity chromatography. Thus, it has been obtained the ultrapurified plasmid vector which can either be used immediately after suspension in saline or lyofilizated and stored at -80˚C. The obtained product has been certified as regards its sterility and toxicity.
Up to now, there have been selected 3 patients with chronic ischaemia of the lower limbs (persistent rest pain and/or unhealed ischaemic ulcers) who cannot undergo interventional or surgical revascularization. For these patients, who are in so-called near-amputation stage, nowadays, there is

no other therapeutical option but amputation. Patients received intramuscular injection of plasmid encoding VEGF165 at 16 sites of the affected limb separated by a distance of 3-4 cm.
After therapy, the patients were further hospitalized for the observation of both the ischaemic symptoms evolution and the possible side-effects of therapy (edema of the cured lower limb, fever, increased levels of acute phase reactants). The only side-effect of angiogenic therapy noticed in these patients was a temporary increase (within the first 6-10 hours) of the acute phase reactant levels, which demonstrates the normal evolution after parenteral administration of the plasmid vectors, resulted from successful penetration into the muscular tissue and the beginning of their local action.
After 1 month, the first 2 patients showed positive evolution with the settlement of rest pain, partial revitalization of the lower limb's cyanotic areas and a mean 5-minute walk distance of about 400 m.
This study is supported by a grant from the Academy of Medical Sciences – VIASAN Programme.

2. HEALTH PROMOTION IN ELDERLY PATIENTS

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ABSTRACT
The **purpose** of the study was to analize the morbidity of this population and to apreciate the way how elderly people benefit form screening tests and desease prevention.
Material and methods: The study was performed on a period of 4 years beginning in 2003 at the Medical Family Departament in collaboration with 7 family medicine doctors, on 3208 patients with age ranging between 65 and 87 years. The study was based on the retrospective revue of the medical recordings and to fill in a special form.
Results: Morbidity analisis: 706 pateints (22%) presented phisical sufferings, cerebral vascular deseases 994(31%), utinary tract infections 451(14%), cardiovascular pathology 1605 (51%),dental problemes 88%,ear and eye disorders 30%,osteophorosis 18%,rheumatic disease 70%.2791 patients (87%) have been instructed regarding the decrease of cardiovascular risc, periodic mesure of blood pressure was performed in 7o%,for 1027 patients (32%) EKG was performed, 1860 (58%) of patients were subjects of glucose blood determination, 481 (15%) were submited to cholesterol leaveles screening for a period less than three years and 1604 (50%) of these patients were instructed about a properly administrated medication.Tests for early stages in prostathic cancer were performed at 21% of men,mamography was prescribed in 0,8% of the wemen involved in this study and for colorectal cancer were screened 5% of the patients.Eldery peoples,age ranging between 65-75 years and high school graduated patients have particulary benefit by the majotity of prorphilactic actiones.
Conclusions:The preventive clinical services and screening tests are insufficient promoted on eldery patients.Health improving and disease prevention stategies have to be implemented on a larger scale by all factores liable for public health.

3. THE INCIDENCE OF B HEMOLYTIC GROUP A STREPTOCOCCI FROM PHARYNGEAL SWABS

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ABSTRACT
Purpose: Streptococci can produce primary infections with various locations, wich are severe due to infective and postinfective complications. The most exposed persons are children, teenagers and young people from crowed communities.
In order to detect the incidence of β hemolytic group A streptococcus (*Streptococcus pyogenes*), in the pharynx, 4607 pharyngeal swabs have been collected during 2005, in a private laboratory from Timişoara.
Material and Method: After performing all the bacteriological stages, investigations have been completed with ASO test (ASLO - serological test), to all patients clinically suspected, including

patients in which β hemolytic streptococcus group A was detected, as well as patients whith negative swab results, but with typical simptoms.
After final lab results, we studied the incidence of poststreptococcal complications, which included 8 patients that have been identified during 2005.
Results: From 4607 collected and performed samples, 3593 (78%) had normal flora, and 1014 (22%) were positive.
From 1014 pathogen species, 117 (11,53%) strains were identified as β hemolytic group A streptococci, 76 (7,5%) were β hemolytic group A streptococci in association with other germs, and the rest of 821 (80,97%) were other pathogenic germs (most frecvent *Staphilococcus aureus*, other groups of streptococci and gram-negative bacilli: *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., *Pseudomonas aeruginosa* etc).
Conclusions: This study detected a high number of β hemolytic group A streptococcus strains - 193 (19,03%), responsible for upper respiratory tract infections. Prevention of spreading of β hemolytic group A streptococcus infections needs a good sanitary education of the population.

4. CHANGING THE REMOVABLE PARTIAL DENTURE METALLIC FRAMEWORK TECHNOLOGY USING "LIGHT CURING" WAXES

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ABSTRACT
AIM OF STUDY: Changing the removable partial denture metallic framework technology is an achievable objective in order to reduce intermediary working stages and use new materials in wax up of metallic framework.
MATERIALS AND METHOD: Materials used for wax up were "LiWa" and "Ti Light" light curing waxes. Were conceived many removable partial denture design choices for different topographical situations. Then, were achieved metallic framework wax patterns directly on the working cast without duplicating and achievement of the refractory cast.
RESULTS: Wax up with "LiWa" is more difficult then that with "Ti Light", first material being more sticky, adherent to instruments more elastic. After light curing these materials, removal of wax pattern from the cast is a delicate procedure because the pattern can crack ("Ti Light" is more breakable then "LiWa".
CONCLUSIONS: Wax up and light curing duration is 25 minutes, so it is more rapid then classic technology, witch is achieved in several hours. In future, materials quality can be improved and the procedure be applied currently.

5. THIN LAYERS FOR IMPROVING THE BIOCOMPATIBILITY OF IMPLANT MATERIALS

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ABSTRACT
The characteristics of the materials employed for implants can be improved by maximizing their biocompatibility and their resistance to chemical corrosion and mechanical wear through the coating with anorganic thin layers, 0.002-0.01 thick, obtained by PVD vacuum deposition.

The materials used as substrates are: 316L stainless steel, Ti5A12 ,5Fe alloy and shape-memory alloy NiTiNb. Thin layers of TiN, TiAlN and multilayered TiN/TiAlNb nitrides were vacuum deposited by the PVD cathodic spring method. Comparative measurement of resistance to corrosion of uncoated and coated materials was performed by measuring the corrosion and critical passivation in physiological solution and artificial saliva currents. Layer composition, microhardness and the adherence to substrate were determined by X-ray difraction. Auger electron spectroscopy, Vickers microhardness and Rockwell diamond scratching adherence test. The biocompatibility of layers was assessed by comparison with uncoated materials according to the Tripan Blue rat hepatocyte culture method.
The objective of this paper is demonstrating the improvement of the biocompatibility, corrosion resistance and superficial hardness of implant materials by thin layer coatings with TiN and TiAlN monolayer and TiN/TiAlN multilayer structures.
The results showed that all explored coatings have a good biocompatibility, show no signs of cytotoxicity and irritating potential. The celular viability was 94.3 % for TiAlN, 03.4% for TiN/TiAlN and 92.6% for TiN. Multilayered TiN/TiAlN had the highest resistance to corrosion, followed by samples coated with TiAlN and TiN. There was no significant difference between layer properties depending on the nature of the substrate.
We conclude that TiN and TiAlN thin layers obtained through vacuum cathodic spring method improve the biocompatibility of implant materials.

6. HIGH PERFORMANCE TECHNICALLY ADVANCED COMPOSITE MATERIALS DESTINED TO BE USED IN THE MEDICAL INDUSTRY

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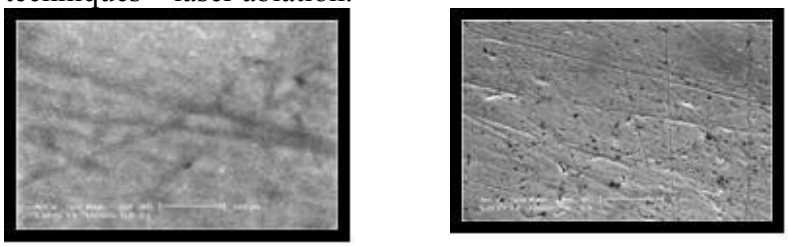
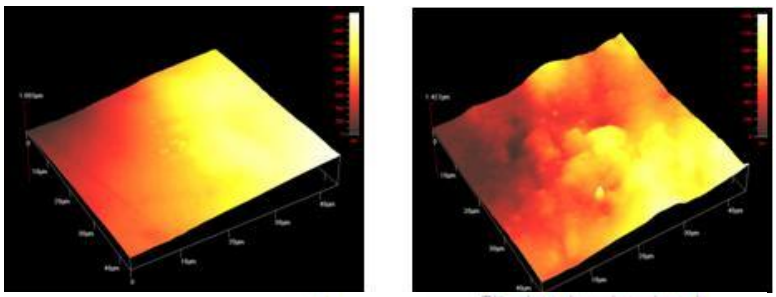
** National Institute of Laser, Plasma and Radiation Physics, Magurele
*** SC BIMETAL SA

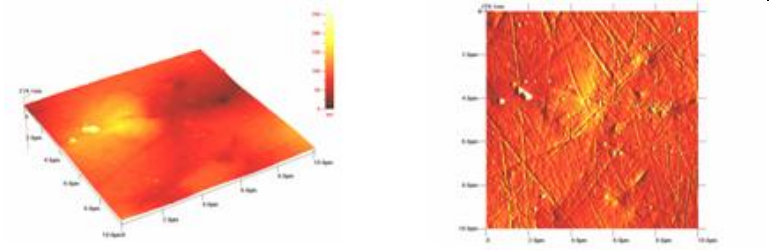
ABSTRACT
Objective: Obtaining porous superelastic materials, to be used in the medical field, from NiTi alloys, and improving the performance of bench-marks obtained by protective and functional covering techniques – laser ablation.



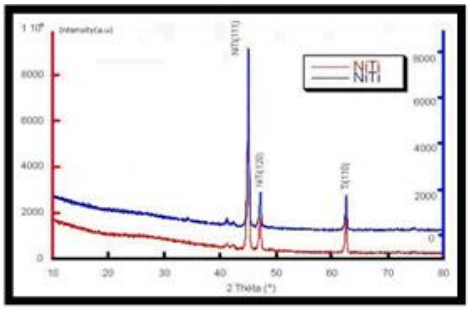
The structure of the nitinol thin layers obtained by laser ablation



AFM images of: a) Ti subtrate, b) NiTi thin layer



AFM images of thin layers of NiTi on microareas: a) si b) – scanning area 10X10 μm;



X-ray difraction on NiTi thin layers/ Ti substrate

Results

- Outstanding porosity at the tissue-implant interface;
- The tissue-implant contact area is 40% greater for Nitinol than for titanium;
- Remarcable biofunctional integration;
- Drastic decrease in the risk of restenosis;
- Offers favourable conditions for cellular growth

Conclusions

- The porous superelastic Nitinol is a highly attractive material in the fields of orthopedics, maxilo-facial surgery and dental implants
- The Nitinol thin layers obtained by laser ablation show homogenous structure and uniform distribution of their components.

7. INTEGRATED OPEN SOURCE HOSPITAL INFORMATION SYSTEM & EHEALTH ENVIROMENT CARDIOPREVENT™

Gabriel Ciorica[1], Silvia Mancas[1], Lucian Stoica[1],Calin Muntean[2], D.M. Duda-Seiman[1], C.A. Sarau[1], Dan Gaita[1], Stefan I. Dragulescu[1]

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ABSTRACT

The e-health project proposed under the label **CARDIOPREVENT™** is a complete open-source, informatic system built around an internet portal providing the necessary web-services, with an open and easily modifiable architecture. These applications are developed under the provisions set by open EHR standards, in a open source environment.

We have realised more relational data-base systems (SQL type) using a collaborative work medium that allows a more pertinent and complete data analysis. We have also included into the system the existent forms (medical files) and used a series of formularies adapted to the project.Data collection is accomplished through the use of the same paramaters of analysis. The developed application is easily deployable to every hospital due to its inclusion of the therapeutical and diagnostic procedures codification and classification according to the ICD 10 standard.

This pilot-project is in current use and development at the Institute of Cardiovascular Diseases Timisoara, Clinical Department of Preventive Cardiology and Cardiovascular Rehabilitation. We have already included more than 3000 patients in the last 24 months, and we use this database for studies, grants, research.

From a common entry-level interface the users are then presented with custom forms for data input or query, according to specific department needs and medical procedures. The DICOM standard is used for medical imaging techniques (X-Ray, CT, angiography etc), providing interoperability with other database systems and medical imaging software. Among other facilities, the user interface includes data-export features for reporting data to local and national healthcare institutions (for epidemiology, statistic analysis, DRG reports).

1. CARDIOPREVENT™ can develop generic, standards-based interfaces between cardiology systems and enterprise systems for scheduling, ordering and results reporting

So far the results we obtained indicate that a multiportal such as **CARDIOPREVENT™** can represent, as it is, an electronic system of multifactorial evaluation in cardiovascular diseases

2. This project needs time though to quantify its penetrability and aplicability, but can represent a model for a standard in Romanian e-health. Currently it is being developed horizontally, vertical development depending on many economical and political decision makers.
3. CARDIOPREVENT™ can providing an implementation framework for open connectivity using *existing standards in preventive cardiology and rehabilitation programs*

8. MAGNETOIMPEDANCE-BASED BIOSENSOR PROTOTYPE FOR SPECIFIC BIOMEDICAL APPLICATIONS

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ABSTRACT
Goal: At present, there are known different detection and bio-detection techniques implemented in biomedical devices such as optical, electrochemical, thermometric, piezoelectric or magnetic. The last one includes the giant magneto-resistance (GMR) and, relative recently, the giant magneto-impedance (GMI) effect. These effects can be used in different GMR or GMI magnetic biosensors that make use of magnetic particles as markers for target biomolecules. In our work we designed and tested a GMI-based biosensor using a special sensing element and in home-prepared or commercially available magnetic micro/nanoparticles.
Material and method. *The sensing element*. Co-Fe-Si-B glass-coated amorphous microwires of 35 micrometers in diameter and 30 mm length was prepared by glass-coating melt-spinning method and used as GMI sensing element. *The magnetic particles.* In home-prepared amorphous microparticles and cobalt nanoparticles, and commercially magnetic beads were used to test their influence on the sensing element. The magnetic amorphous microwire was arranged parallel to an external d.c. magnetic field. The magnetic particles were dispersed around the microwire. An external magnetic field has been applied on the microwire that is passed by an a.c. current.
Results. Different magneto-impedance variations were obtained when using amorphous microparticles with different sizes. Moreover, the sensing element was sensitive even when using magnetic nanoparticles. As for example an important magneto-impedance variation of about 40 Ω was obtained when using amorphous magnetic microparticles.
Conclusions. The magnetic biosensor prototype presents high sensitivity and it can detect both magnetic micro/nanoparticles and commercially magnetic beads. Therefore, such a magnetic detection system can be used successfully in magnetic bio-detection applications for specific biomolecule identification.
This work was partially supported from the contract CEEX no. 42/2005 RO-NANOMED.

9. EMPLOYMENT OF TITANIUM ALLOYS IN THE MANUFACTURE OF SOME TYPES OF SPINAL IMPLANTS

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*** National Imstitute for Research and Development of Microtechnologies – Bucharest;
**** Academy for Economical Studies - Bucharest

ABSTRACT
Objective. Obtaining and characterizing from a functional, structural, dimansional and material point of view, Ti and Ti alloy spinal implants
Materials and methods. The Ti6Al4V alloy has been widely used as implant material, but its current usage is restricted due to Vanadium toxicity. Thus, the Ti6Al7Nb alloy was perfected by replacing V from the Ti6Al4V alloy with the equivalent quantity of Nb. The resistance of the Ti6Al7Nb alloy is 933 Mpa, a little less than that of the Ti6Al4V alloy. The Ti6Al7Nb alloy is less corrosive and its resistance to corrosion is close to that of the comercially available pure titanium. The rigidity of the implant and the characterstics of its fastening on the bone tissue are important factors of bone

remodelling. Consequently, the Ti6Al7Nb alloy conveniently meets both qualities of mechanical resistance and biocompatibility.
In order to clarify the functional, structural, dimensional and material properties of the implants, the following scientific research activities will be initiated: synthesis of the alloys; structural, mechanical and biocompatibility tests; designing and obtaining of the implants. To optimize the results mathematical modelling of structure-properties relations and of implant functionality will be performed.

Results and conclusions.

This project will be finalized with the obtaining of spinal implants that will satisfy the following requirements:

- Monoaxial pedicle screws, with a diameter from 4,5 mm to 65 mm
- Pedicle hooks, laminar hooks, transverse hooks;
- Fixing nut, fixing rod of 5 mm with a length of 200 to 650 mm

The range above comprises all necessary dimensions and types of surgical implants for all categories of patients.

10. MICROSENSORS AND ACTUATORS FOR MICRO-OPTO-ELECTROMECHANICAL SYSTEMS MANUFACTURED BY SHAPE MEMORY ALLOY NANOENGINEERING

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ABSTRACT

Goal: The main goal of the project refers to the fabrication of microsensors and actuators based on performant shape memory alloys, materialized through secondary objectives like: fabrication of nanoengineered shape memory alloys and characterization of materials behavior and manufactured products in various environments.

Materials and method. The focus is on Cu-Zn-Al and Cu-Al-Ni alloys and the biocompatibility tests have been oriented toward NiTi bulk materials. The basic method used for achieving the goals was based on the "melt-spinning" technique and the aim was to generate alloys with favorable texture, thus leading to shape memory effect with larger amplitude.

Results:

Copper based pre-alloys have been manufactured based on Cu-Zn-Al and Cu-Al-Ni families, following several steps, like:

- design and implementation of the technology for rapid solidification and heat treatment;
- characterization by optical and X-ray diffraction techniques;
- optimization based on analysis of as cast and quenched structures.

A system for ribbons fabrication has been optimized (especially on what concerns the control of the wheel rotation and ejecting temperature) and shape memory alloy ribbons with a width between 2.5 and 4 mm and 30 to 50 μm thickness have been produced.
Assembling technologies for shape memory alloy ribbons and bulk materials have been developed for similar and dissimilar materials as well as for ribbon joins.
Microsensor and actuator demonstrative products have been designed and produced, based on the manufactured materials and the NiTi behavior in biocompatible environments has been studied.

Conclusions. The possibility to fabricate better quality ribbons and to use them to design prototypes for new applications has been demonstrated and a technique to assess the shape memory alloy biocompatibility has been analyzed.

11. NANOCONTROL AND MULTIFUNCTIONALITY IN MATERIALS, MICROLAYERS AND ARCHITECTURES WITH SHAPE MEMORY PROPERTIES

Corneliu M. Craciunescu[1,2], Victor Budau[2], Iona Mitelea[2], Mihaela Valeanu[3], Horia Chiriac[4], Aurel Ercuta[5], Valentin Bardean[6], Virgil Paunescu[7], Dieter Schinle[8], Mihaela Stoica[9]

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[2] Politechnics University Timisoara
[3] National R&D Institute for Materials Physics Bucharest
[4] National Physics R&D Institute Iasi
[5] Western University Timisoara
[6] National R&D Institute National for Welding and Material Testing
[7] University of Medicine and Pharmacy Victor Babes Timisoara
[8] Machines Building Factories Resita
[9] National R&D Institute in the Laser, Plasma and Radiation Physiscs Bucharest

ABSTRACT

Goal. The main goal of the project refers to the analysis of the shape memory alloys functionality with focus on new ferrous and ferromagnetic alloy families, as well as on the use of these materials in performant structures and architectures.

Material si metoda. The main alloy families cicaonsidered are ferrous shape memory alloys (Fe-Mn-Si, Fe-Ni-Co-Ti as well as FeCrNiMnSiCo stainless alloys), Heusler-type alloys (Co-Ni-Ga, Ni-Mn-Ga, Co-Ni-Fe, Co-Ni-Al, etc.), and NiTi family, respectively. The basic methods proposed are those through which particular materials will be manufactured as ribbons, glass coated wires and suction solidified bars. The characterization methods used are complex, based on thermal, magnetical, magnetoelastic, biological techniques, and the finality is related to the fabrication of advanced materials.

Results. Bulk ferous shape memory alloys have been manufactured so far by arc melting and subsequently rapidly solidified ribbons ($FeMn_{32}Si_6$) have been produced using the melt-spinning technique. The ribbons have been vacuum heat treated, and as result the shape memory effect has been observed above room temperature and as a function of the applied heat treatment they also becomed magnetic.

Other ribbons (Fe-Ni-Co-Ti) showed the shape memory effect at liquid nitrogen temperature. Preliminary investigations by electrical analysis showed anomalies in the behavior of Fe-Mn-Si alloys, in the vicinity of 0- 50 K, that has been attributed to a phase transition.

Conclusions. Promising research results have been obtained on shape memory alloys, based on thorough analysis and ferrous shape memory alloy bulk and ribbons, with shape memory properties and – as a function of composition – magnetic or stainless properties.

12. FACTOR ANALYSIS OF PULSE WAVE VELOCITY INCREASE IN ASYMPTOMATIC SUBJECTS

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ABSTRACT

Objectives: Insulin resistance (IR) is an essential feature of the metabolic syndrome, but the association IR – endothelial dysfunction is still disputed. There is scientific proof regarding the relation carotid-radial pulse wave velocity (PWV-CR), endothelial function and cardiovascular risk. The work proposes to verify the hypothesis that PWV-CR is increased in subjects with IR vs. without IR.

Methods: PWV-CR was assessed using the Complior device in order to observe its behavior in asymptomatic subjects with (n=44) and without IR (n=14).

Results: The association IR and blood hypertension was present in 65.51% from the subjects, IR and left ventricular diastolic dysfunction in 63,79%, IR and TG/HDL≥3.5 in 56,89%, IR and LDL>100 mg/dL in 46,55%. In the group with IR, PWV-CR had a mean value of 10.83±2.52 m/s. Increased

PWV-CR correlated significantly with IR (p=0.004), with increased TG levels (p=0.01) and extremely significant with the TG/HDL≥3.5 ratio (p=0.0000001). The correlation with LDL was not significant.
Conclusions: (a) PWV-CR was with 13.85% higher in the IR group and with 8.77% higher in subjects with metabolic syndrome. (b) It is possible that this vascular dysfunction to be present early in subjects at risk of developing metabolic syndrome. (c) The TG/HDL≥3.5 ratio was the best IR predictor.

13. THE OSTEOGENIC DIFFERENTIATION OF CANINE MESENCHYMAL STEM CELLS IN COMPOSITE MATRICES

Erika Deak[1], Mihai Palko[2], Simona Anghel[1], Tiberiu Popescu-Negreanu[3], Cornel Igna[2], Gabriela Tanasie[1]
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[2]Department of Surgery, University of Agricultural Sciences and Veterinary Medicine Timisoara,
[3]"Sticla si ceramica PONETI" Ltd

ABSTRACT
Tissue engineering based on stem cells involves the exploration of two main characteristics of these cells: the high proliferation capacity and the pluripotency.
Aim of the study: Investigation of in vivo proliferative and differentiation potential of canine mesenchymal stem cells cultured on composite matrices.
Materials and methods: The mesenchymal stem cells were isolated from bone marrow aspirates (harvested by humerus punction) from 5 healthy dogs. The harvesting procedures were performed safe and sterile according to the approval of university ethics committee. For cell isolation we used the plastic adherence method. The cells were cultivated on semiconfluence, trypsinised and seeded in DMEM+10% FCS on a density of 10^5cells/cm^2. At second passage the cells were seeded on 3D composite matrices, 10^6 cells/matrix. The media was supplemented in osteogenic factors (beta glicerophosphate, ascorbate, dexametasone). We counted the unadherent cells (trypan blue technique) and after 14 days we checked for bone mineralization using von Kossa staining.
Results: Canine mesenchymal stem cells showed a characteristic, fibroblastoid morphology, similar with human mesenchymal stem cells. These cells can be relatively easy differentiated into osteoblasts in vitro, using the appropriate media supplementation. In vitro, the cells had a good adherence on composite matrices. The ex vivo examination of mixed implants (biovitroceramics-colagen-mesenchymal stem cells) proved the mesenchymal stem cells differentiation into osteoblasts.

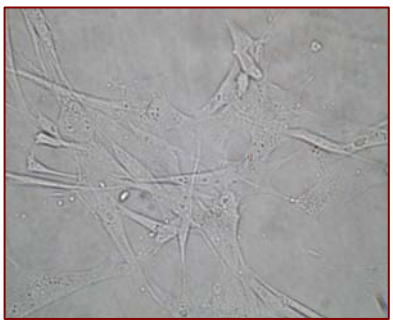
Fig. 1. Canine mesenchymal stem cells, passage 1, ob. 40x

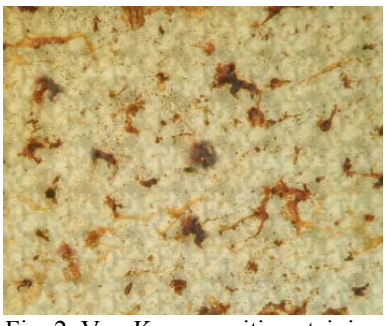
Fig. 2. Von Kossa positive staining

The study was supported by national excellence grant CEEX 45/2005 (CELLART)

14. INFLUENCE OF IMMOBILISATION METHOD ON THE KINETICS OF PROTEASES ENTRAPPED IN NANOPOROUS SILICA GELS OBTAINED USING THE SOL-GEL TECHNIQUE

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ABSTRACT
Aims: The sol – gel process is a method allowing the entrapment of functionally active biomolecules in inorganic silica at room temperature. The kinetic constants for immobilized enzymes are usually different from those for the native form, as conformational changes, steric hindrances and partition and diffusion effects may occur on enzyme immobilization. The conformational effects may also change the affinity between enzyme and substrate. This is not always a disadvantage, because the porous silica network may protect proteins against unfolding.
The aim of this work was the immobilisation of two proteases, a commercial product, Alcalase, and a protease preparation obtained by submerged fermentation of *Bacillus licheniformis* B 40 local strain. Two immobilisation methods were used: entrapment in silica gels and entrapment-deposition on a inorganic support. The enzyme-substrate affinity modification was investigated based on the kinetics of native and immobilised enzymes respectively.
Materials and methods: The general immobilisation method used tetraethoxysilane (TEOS) as gel precursor. The sol-gel synthesis was done in two steps, by acid (pH 3.5-4.0) and base (pH 7.5-8.0) catalysis. A local ceramic material (provider Procema SA) was used as co support for entrapment-deposition. The kinetics of the native and immobilised enzymes was studied in the hydrolysis reaction of a model substrate, a low molecular mass ester, para-nitrophenyl acetate.
Results and discussion: Alcalase immobilisation provided entrapped products with 10 time's higher protease activity and better immobilisation yields, compared with the *Bacillus licheniformis* B 40 enzyme. For both enzymes, the *Michelis constant* K_m and the *maximum rate* V_{max} of the immobilised enzymes were higher than those of the soluble ones. The *Bacillus licheniformis* B 40 enzyme has kinetic parameters comparable with those of the commercial enzyme.
Conclusions: For both enzymes, the hydrolysis reaction follows a Michaelis-Menten type kinetic. Immobilisation changes the kinetic parameters. V_{max}/K_m ratio suggests that, by immobilisation, the catalytic efficiency increases, thus compensating the partial decrease of the enzyme-substrate affinity.

15. IMPORTANT STRUCTURAL DESCRIPTORS TO DEFINE BIOLOGICAL ACTIVITY AND MOLECULAR MODELING TO DESIGN NEW ANTI-HIV DRUGS

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ABSTRACT
Aim: to perform a QSAR (quantitative structure-activity relationship) study using molecular modeling methods upon a series of 79 HEPT ligands which act as HIV reverse-transcriptase (RT) inhibitors in order to map the space of the interaction site, as well as to assess structural parameters important to define biological activity.
Materials and methods: Molecular modeling was performed using the HyperChem 7.01 program and conformational analysis using Conformational Search. The most stable conformations were identified in the series with 79 HEPT ligands and they were used to obtain the hypermolecule in order to perform a classic MTD method. A large number of structural parameters was assessed with the Dragon program and their predictive power was tested with correlation methods.
Results: Biological active molecules are less symmetrical shapes. The obtained multiparametric models with various structural descriptors led to correlation coefficients of 0.9.

Conclusions: Beyond the possibility to identify and to remove from the beginning those molecular structures which don't show any therapeutic potentiality, well designed QSAR studies allow to collect essential structural data necessary for an increased biological activity, suggesting certain requirements for an increased anti-HIV effect.

16. CARDIOVASCULAR RISK: ENDOTHELIAL DYSFUNCTION, INSULIN RESISTANCE AND ASSOCIATED FACTORS

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ABSTRACT
Objectives: Epidemiological evidence shows that smoking increases the incidence of fatal and non-fatal cardiovascular events through insulin resistance (IR). The work proposes to assess the relation between smoking, IR and endothelial dysfunction in asymptomatic hypertensive men.
Methods: We assessed the smoking state (pack years), carotid-radial pulse wave velocity (PWV-CR) with Complior and IR (IRHOMA) in asymptomatic hypertensive men.
Results: 42 asymptomatic hypertensive men, mean age 57.57±16.4 years: 26 (61.9%) with PWV-CR≥9.5 m/s and 28 (66.66%) with IRHOMA≥1. Waist circumference has a positive correlation with the state of IR, especially in those with PWV-CR≥9.5 m/s and LDL>100 mg/dL (R=0.59, R^2=0.34, p=0.004). The odds to have IR are 2.75 if TG/HDL≥3.5 and the relative risk in this case to develop IR is 1.38. The odds to have PWV-CR≥9.5 m/s are 5.40 in subjects with IRHOMA≥1 and the relative risk to develop increased PWV-CR is 1.85 when IRHOMA≥1. Smoking amplifies significantly the PWV-CR increasing level (p=0.0003).
Conclusions: (a) Smoking is a public health problem because of the consequences on the vessels quality. (b) IR plays a key role in cardiovascular risk assessing in middle-aged asymptomatic men. (c) Adding other modifiable factors to IR, the vascular impact is aggravated. (d) The cost-effectiveness of prevention programmes is in favor of a healthy lifestyle adopted as early as possible.

17. IMPAIRED ENDOTHELIAL-DEPENDENT VASORELAXATION IN ISOLATED HUMAN MAMMARY ARTERIES PREINCUBATED WITH TRIGLICERIDE-RICH REMNANTS LIPOPROTEIN

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ABSTRACT
Several studies have now shown an impairment of endothelial function in association with hypertriglyceridemia. Major changes in lipoprotein metabolism occur in these individuals, resulting in the formation of triglyceride-rich remnant lipoprotein, small and dense LDL (predispose to peroxidation), and decreased HDL-cholesterol. We examined "in vitro" the effect of remnant lipoproteins on acetylcholine (Ach)-induced endothelium-dependent relaxation, and sodium nitroprusside (SNP)-induced endothelium-independent relaxation in human mammary arterial rings. Our results suggest that preincubation with triglyceride-rich remnant lipoproteins inhibited endothelial-dependent vasorelaxation (maximal relaxation expressed as percentage was 34.15 ± 4.88 in remants group versus 49.03 ± 9.85 in control group, p=0.008) but did not affect endothelium-independent vasorelaxation (maximal relaxation was 93.33 ± 7.36 in remnants group, versus in 95.86 ± 3.48 control group, p=0.46). This endothelial dysfunction induced by remnant lipoprotein could contribute to the pathogenesis of atherosclerosis associated with hypertriglyceridemia. This conclusion supports the concept that each of the changes in the plasma lipoproteins associate with elevated triglyceride plasma level contribuite to the increased risk of premature cardiovascular disease.

18. INTERFACE MECHANICS AND HISTOLOGY OF TITANIUM AND HYDROXYAPATITE-COATED TITANIUM FOR MEDICAL IMPLANT APPLICATIONS

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ABSTRACT
Objectives: in-vivo testing biocompatibility of implants coated with osteoconductive supports in femoral assembling on canine biological model; clinical, radiological and histological studies to establish adhesion between biological support and implant and bone contact reaction checking (presence of osteogenic and osteoclastic cells, presence of blood vessels and preosseous matrix formation).
Experimental model: eight types of titanium lamellar implants (osteosinthesys plate type, PCT, AO model, dimensions between 10-18 mm made by Aesculap that have the internal face (bone contact face) coated at INCDSIM Timisoara with various concentrations of titanium powders, hydroxyapatite and titanium-hydroxyapatite combinations were fixed into the femoral cortex of five dogs (common breed, females, three year old and 18 kg bodyweight) using a standard aseptically technique.
Methods: *Clinical study* – behavior changes, central body temperature and body weight were followed and recorded on all the experimental period.
Radiological examination of the femours with implants was made by *cranio-caudal* incidence radiographs, using *65 kVp and 20 mAs*.
Macroscopical examination – *the femours with implants were prelevated and all macroscopical changes noted.*
Microscopical examination – the pieces were demineralized (immersion in 10% tricloracetic acid) and histological processed by Hematoxylin-Eozin and Mallory tricromic coloration.
Results: The adhesion of bone support to implants is better on those coated with hydroxyapatite that at the ones with titanium powders. The macroscopical changes reveal the biocompatibility with the canine bone support of the implants in study. The biological changes radiographic observed around the implants from the second experiment are placed in the normal, physiological evolution of bone support under these kind of manipulation, which reveals a perfect biocompatibility. The active biointerface behavior was observed on implants coated with hydroxyapatite and combination of porous titanium and hydroxyapatite, on which the histological changes observed were osteoinduction and osteoconduction phenomenons.
Clinical relevancy: The implants coated with hydroxyapatite have biocompatibility with canine bone support and also have osteoinductive and osteoconductive capacity which makes them recommended for clinical testing.

19. FLOWCYTOMETRIC MEASUREMENT OF BIOCOMPATIBILITY OF MULTILAYERED NANOSTRUCTURES DEPOSITED BY MAGNETRON REACTIVE ATOMISATION

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ABSTRACT
The study of the biocompatibility of implant materials can be accurately performed by using the calcein-AM flowcytometry.

The materials used as substrates were 316L stainless steel and Ti6A14V alloy. The multilayered nanostructures $(Ti/TiN/TiAlN)_n$, n being the total number of layers, were vacuum deposited by magnetron reactive atomisation, as it results in a minimal surface rugosity. Comparative measurement of the biocompatibility of uncoated and coated materials was performed using the Calcein-AM test, the best indicator of cell viability due to its high retention inside the cell and relative insensitivity of the fluorescence to pH values within physiological boundaries. Calcein, the hydrolysis product of Calcein-AM, a polianionic derivate of fluoresceine, has 6 negative and 2 positive charges at pH 7.0. The activity of cellular esterases, resulting in an increased fluorescence, is a degree of cellular viability. Layer composition, microhardness and the adherence to substrate were determined by X-ray difraction. Auger electron spectroscopy, Vickers microhardness and Rockwell diamond scratching adherence test.
The objective of this paper is demonstrating the improvement of the biocompatibility, corrosion resistance and superficial hardness of implant materials by thin layer coatings with multilayered nanostructures of $(Ti/TiN/TiAlN)_{n=100}$ with reduced surface rugosity.
The results showed that all explored coatings have a good biocompatibility. Calcein, as a hydrolysis product of Calcein-AM, confers the cells a high degree of fluorescence related to cellular viability as this compound is only retained by cells that have an intact membrane. Cell viability was 49% for the Ti6Al4V alloy, 42% for 316L stainless steel, 75% for$(Ti/TiN/TiAlN)_{n=100}$, 65% for TiN and 63% for TiAlN.
We conclude that the Calcein-AM flowcytometry test is a fast and sensitive method for assessing the biocompatibility of implant materials.

20. THEORETICAL ANALYSIS OF SEQUENCE-STRUCTURE RELATIONSHIP FOR CALCIUM BINDING PROTEINS

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ABSTRACT
Aim : theoretical analysis of intramolecular interactions responsible for tertiary structure folding of calcium binding proteins belonging to the "E-F hand" family and prediction or tertiary structure of centrins.
Method:
- sequence alignment for proteins under study ;
- prediction of the tertiary structure using Mitaku's server ;
- determination of net charge of entire protein and those of their N and C-terminal domains;
- determination of the ratio of charged amino acids and that of hydrophobic amino acids both for the entire protein and for its domains.

Results. Sequence similarity is high (more than 60%) for the extended structures and quite small for compact structures (less than 26%). Centrins have a high sequence similarity with extended structures. Theoretical analysis of physicochemical properties of amino acids belonging to these proteins shows that extended structures are abundant in charged amino acids (more than 30%) in both domains. They also have a high negative net charge. Compact structures are abundant in hydrophobic residues, especially in the central linker. Average hydropaticity is higher for compact structures than for extended ones.
Conclusions. The high ratio of charged residues and high negative net charges of domains confirm the hypothesis that electrostatic repulsions are responsible for great stability of extended structures. In the case of compact structures, the high ratio of hydrophobic residues confirms that hydrophobic interactions contribute significantly to the stability of these proteins. Comparison of the physicochemical properties of amino acids belonging to centrins sequences show that human centrin

3 and yeast centrin could adopt a compact tertiary structure and human centrins 1 and 2 could adopt extended tertiary structures.

21. THE IMPORTANCE OF PULSE WAVE VELOCITY IN HYPERTENSIVE PATIENTS WHICH ARE TO BE INCLUDED IN A REHABILITATION PROGRAM.

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ABSTRACT
Introduction: The pulse wave velocity (PWV) represents a method of evaluating the arterial stiffness. Recent epidemiological studies have proved that the PWV and the pulse pressure (PP) represent independent predictors of cardiovascular mortality in hypertensive old subjects. The change of arterial rigidity appears in initial states of arterial hypertension, and this is why the early identifying of PWV values could allow the identifying of and intervention on the complications of hypertensive disorder.
Methods: We studied 89 subjects, with average age of 55.22±11,69 years; of which: men: 47.19% and women: 52.81%, with essential hypertension: normal high, grade 1 and 2 (ESC-ESH 2003). The subjects have been evaluated from a clinical and paraclinical point of view according to the standard protocol of inclusion in the recovery program. The PWV of the carotid-radial (PWVc-r) has been determined for all subjects through automatic monitoring using the Complior device.
Results: We have noticed that the following cardiovascular risk factors (CVRF): male aged over 55 associates prevalently with values of PWVc-r over 10 m/s (bivaried analysis through the chi square test: OR=3.11; 95%CI: 1.20-8.07), the same correlation has also been obtained for smokers with PWVc-r values >10 m/s (OR=3.39; 95%CI: 1.13-10.20). There is also a simple linear correlation between PWV (the dependent variable) and the following independent variables: the systolic blood pressure (SBP) and the pulsed pressure PP (r=0.24, p=0.027).
Conclusion: Evaluating the arterial stiffness by measuring the PWV may constitute a reference in the evaluation of the benefits of the cardiovascular rehabilitation program for a hypertensive patient. Follow up data reviles: the arterial rigidity correlates positively with some CVRF: males aged over 55, as well as with the condition of being smokers. The SBP, as well as the PP values modify the arterial stiffness parameters like PWV.

22. GETTING THROUGH ALTERNATIVE METHODS OF DOPED TiO2 NANOCRYSTALS WITH METALICS IONS.APLICATIONS STUDY FOR HEALTH, BIOLOGY AND ENVIRONMENT

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ABSTRACT
Purpose. The purpose to this project is getting the undoped and doped TiO2 nanocrystals with Au and Ag ions through sol-gel method and hydrothermal method attend ultrasonic/microunde.The interest crystallization form of nanocrystals is anatas and rutil, and medium dimensions of the particles are to maximum 30nm.The titan dioxide doped with metallic's ions get through alternative methods(sol-gel, hydrothermal) offer an large aria of photocatalitics applications in medicine, environment protection and agriculture, opening new perspectives for technical performances of the devices with solid body.
Materials and methods. The precursors used in synthesis of TiO2 doped with metallic's ions were titan izopropoxid, titan tetrachloride, etilic alcohol, oxalic acid, distillate water, silver azotate and tetracloroauric acid (dopaded precursors). The used methods in titan dioxide synthesis were sol-gel and hydrothermal method. The field of baking and autoclave temperatures was between 200^0C and 600^0C.

Results:

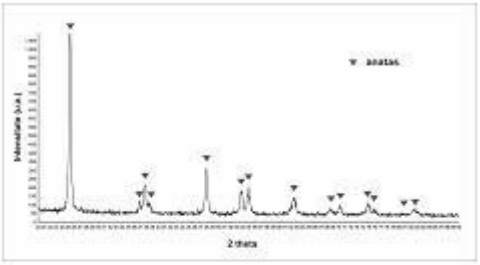

Fig 1. Diffraction spectrum of X razes of TiO2 – undoped – sol-gel

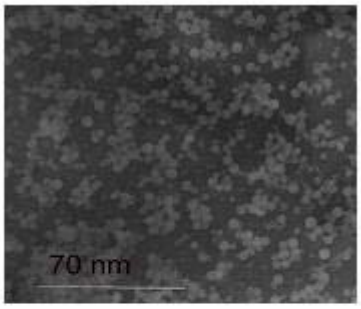

Fig.2.TEM image for TiO2 – undoped – sol-gel

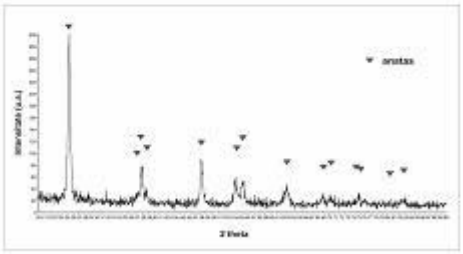

Fig.3. Diffraction spectrum of X razes of TiO2 – doped with Au – sol-gel

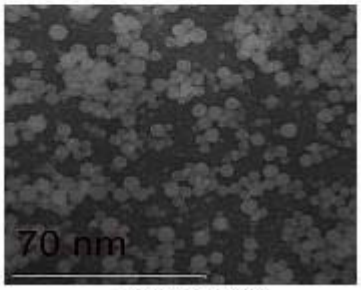

Fig.4TEM image for TiO2—doped with Au – sol-gel

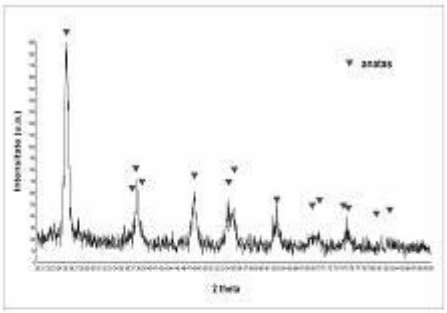

Fig. 5. Diffraction spectrum of X razes of TiO2 – undoped –hydrothermal

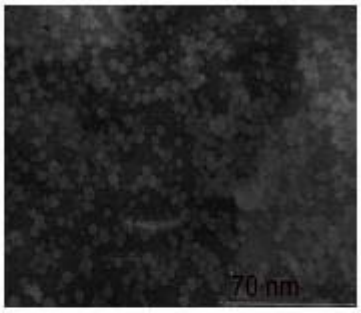

Fig.6. TEM image for TiO2— undoped – hydrothermal

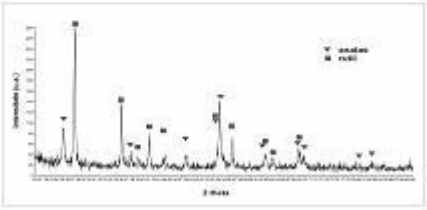

Fig.7.Diffraction spectrum of X razes of TiO2 – doped with Au - hydrothermal

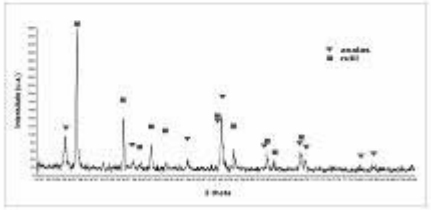

Fig.8. Diffraction spectrum of X razes for TiO2 – doped with Ag - hydrothermal