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CONTENTS

1. CLINICAL GRADE AND GMP PRODUCTION OF MSC

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The concept of progenitors/stem cells for cells of mesenchymal origin, which led to the description of mesenchymal stem cells (MSCs), arose during the 1970s with Friedenstein and colleagues [1]. Since the 1990s, increasing experimental data has allowed for the definition of MSCs as multipotent adult stem cells that can differentiate into connective skeletal tissue, bone, cartilage, marrow stroma, and adipocytes [2-4]. In addition, controversial data suggest that MSCs may give rise to sarcomeric muscle (skeletal and cardiac) [5-7], endothelial cells [8], and even cells of nonmesodermal origin, such as hepatocytes [9], neural cells [10], and epithelial cells [11,12].
MSCs have immunoregulatory actions and interact with all the cells involved in immune response [13], different molecules or cytokines have been shown to play a role in this immuno-regulatory action [14,15]. However, some controversial studies argue that MSCs could be immunogenic in immuno-competent mice suggesting that these cells are not intrinsically immuno-privileged [16]. The wide range of differentiation potential of MSCs, the possibility of their engraftment [17], their immunosuppressive effect [18], and their expansion through culture led to increasing clinical interest in the use of MSCs, through intravenous infusion or site-directed administration, in numerous pathologic situations. Since the first phase-1 study that demonstrated the good tolerance and safety of MSC transplantation [19], clinical trials have investigated large bone defects [20], genetic bone disease as osteogenesis imperfecta [17,21], haematopoietic stem cell transplantation for repair of haematopoietic stroma [22], treatment or prevention of graft versus host disease [23,24]. During the repair process of damaged tissues, the effects of MSCs can be related to different mechanisms, differentiation towards tissue specific pathway, repair

of microenvironment with paracrine/juxtacrine effects by growth factors and cytokines [25] or extracellular matrix reorganization [26].

To perform clinical trials billions rather than millions of MSCs, solely or linked to biomaterials, are needed. Producing MSCs for that purpose necessitates adhering to good manufacturing practices (GMP) to insure the delivery of a «cell drug» that is safe, reproducible, and efficient. However, standards for the production of GMP-grade MSCs in clinical trials are lacking. All parts of the process must be defined, specifically

1. *Starting material*: donor, tissue origin, separation or enrichment procedures…
2. *Culture processing*: cell density, number of passages, culture medium
3. *Devices for cell culture*: To reach the GMP goal, cells need to be cultured in as close to a closed system as possible. Analytical methods are needed to assay the active compound and impurities.
4. *Quality control (QC)*: eligibility of the donor must be considered and standards for phenotype, functional potential, microbiological safety defined. Last but nor least, QC has to check the absence of transformation during the culture process.

STARTING MATERIAL FOR CULTURING MSCS

Since the first description of colony forming units fibroblats (CFU-Fs), the main source of MSCs remains bone marrow [20-24]. The age of the donor is important, and bone marrow of children contains a higher concentration of CFU-Fs than that of adults [27]. MSCs are present in the mononuclear fraction of bone marrow cells [4,19-24]. After being separated by density gradient, most of the MSC populations are isolated because of their physical property of plastic adherence [4,19-24]. Cultured MSCs express a number of markers, none of which are specific individually or in combination [28-33]. Before culture, populations can be selected for enrichment in MSCs with using different expressed Ag STRO-1^{+}, □1 integrin subunit (CD49a), CD 271 [34, 35, 36]. As a consequence, immuno-selection could lead to a loss of the most primitive progenitors/stem cells [37]. There are now potential other sources of MSCs. Although the topic has been controversial for years, recent data have shown that umbilical cord blood is a potential source [38-40]. MSCs from cord blood can be isolated following a technique close to that used for bone-marrow MSCs, the critical parameters being time from collection to isolation, volume of cord blood, and amount of mononuclear cells [39]. Finally, adipose tissues may represent an important source of easily available and abundant MSCs [41-43]. The cells of this tissue has already shown very interesting properties of endothelial and cardiac differentiation both *in vivo* and *in vitro* [9,49]. Some other sources such as trabecular bone (44) or amniotic membrane could have a clinical interest.

MSC CULTURE PROCESSING

Different parameters must be mastered for achieving GMP-grade MSC culture.

Cell plating:

Cell plating density is a critical parameter to ensure good expansion rate and maintenance of the differentiation potential of MSCs. The team of Prockop demonstrated that the development of early progenitors/stem cells as RS cells depended on very low plating density [37,45].

Passages:

MSCs are adherent cells that grow on plastic and have normal growth inhibition at confluence leading to the use of successive passages for obtaining large amounts of pure MSCs devoid of any other cells. If further passages are necessary, these can alter the quality of MSCs. In humans, after the first 3 weeks of initial culture and 12 to 15 doublings, successive passages slow the proliferation rate, and cells progressively show loss of multi-potentency [46,47]. These alterations are more pronounced with material from adults than from children [27].

Culture medium:

This is the final main parameter to consider in GMP-grade MSC culture. The basic composition does not seem to cause a problem, and DMEM or □MEM are commonly used. The two pivotal compounds in medium composition are - serum derived from animal or human origin (FCS versus human serum or plasma) and growth factors.

Classically, the optimal conditions for MSC expansion require FCS supplemented media, the standard being 10%. This FCS needs to be carefully tested to ensure the best expansion rate [4,21,37]. Although FCS carefully tested for viruses and ensuring good traceability exists, the risk of transmission of

infectious disease cannot be excluded. Moreover, in such a culture medium, MSCs retain in their cytoplasm some FCS protein [48] that may elicit immunologic response *in vivo* [21,48]. Some teams try to replace FCS with human autologous serum or blood group AB serum that both are less efficient, but supplementing the medium with human AB serum by FGF2 overcomes this deficit [49]. As demonstrated by different teams, the use of platelet growth factor enriched human plasma (PGFEP) represent an efficient alternative to FCS (50,51). But, MSC cultivated using PGFEP could have changed in their functions as previously shown for osteogenic differentiation potential [52]. Using different cultures conditions should be followed by careful quality control of the processed MSCs.

The growth-factor requirement of MSCs is not completely known: it includes at least platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor □(TGF□), insulin-like growth factor, and FGF2 [53-55]. The use of FGF2 allows for better expansion efficiency, preserved differentiation potential, and decreased the time of culture for the same number of cells [56,57]. FGF2 is used to produce cells in an ongoing approved French national trial [58].

Supplementation with exogenous growth factors can increase the expansion rate and are mandatory in serum-free media. Some serum-free media have been developed in the research process, but such a medium for clinical-scale production according GMP is still awaited.

The characteristics of surface could interact with the characteristics of MSCs, this is demonstrated for the matrix elasticity acting directly on the differentiation pattern [59] Moreover, culture surfaces have to be improved for allowing a better attachment, improved growth, and an easy way to deliver and transfer the cells to specific tissues.

DEVICES FOR MSC CULTURE

Today, totally closed systems for MSC culture are not yet available, but GMP conditions can be reached. To perform MSC culture in a near-closed system, specific connecting systems with bags containing medium were developed by Macopharma (Tourcoing, France) in association with EFS.

QUALITY CONTROLS

The control of the harvested graft ensure that the graft contains quality cells and a sufficient number to reach the necessary number of produced cells and that it is not likely to transmit infectious disease. The number of native MSCs within BM could be assessed by testing CFU-F or by flow cytometry (60). As recently emphasized, it is imperative to implement accurate controls to take into account some of potential dangers in the uses of cultivated MSCs (e.g. transformation, and immunosuppressive activities) (61).

As shown recently, transformation of human MSCs can occur in cells cultured for a long time (62). Moreover, Ewing tumors have been demonstrated to evolve from MSCs (63). Often, in human as in mice (62,64), during the transformation process, chromosome abnormalities involving the locus of c-myc are found. It will be of importance to karyotype the final cell product, but also to develop more sensitive analysis at genomic/transcriptomic level.

The immuno-suppressive properties of MSCs have already been exploited for the treatment of acute graft-versus-host disease (aGVHD) after allogeneic stem cell transplantation (23,24). In addition, standardized and fully-validated methods are urgently needed in order to compare the immunological properties of MSCs obtained from different sources in different culture conditions. In allogeneic settings, it will be of importance to test all aspects of the interactions of MSCs with the immune system.

FUTURE IMPROVEMENTS

The basic parameters for cultivating MSCs are described, the main immunological functions and differentiation pathways of MSCs are known, and basic controls of cultivated MSCs are widely used. But, consistent GMP-grade processing of MSCs, namely derived from different sources and tissues, reaching the quality for cellular therapeutics are still lacking because of lack of standardisation.

The major critical points of the entire production process are the choice of starting materials, the culture process (enrichment/purification of starting material, media and surfaces), and the quality and safety controls of final products. To reach these targets we have:

- To develop innovative specific GMP culture processes adapted to different clinically available sources of MSCs.
- To test the efficiency and safety of GMP-grade MSCs in relevant *in vivo* and *in vitro* models..

- To define standards for specific and appropriate controls of cultivated MSCs, particularly to assess the immune-regulatory function of MSCs, and the absence of transformation.

2. STEM CELLS AND OSTEOARTICULAR REGENERATION

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ABSTRACT

It is well known that structures as bones and cartilages have a limited self-repair capacity. Generating new tissue engineering methods, which allow the replacement of organs through ex vivo culture of autologous stem cells, may represent a valid substitute for organ transplant. The activities performed as part of this study were pursuit successively the following:

- Isolation and cultivation of animal adult stem cells
- Elaboration of technologies in order to obtain scaffolds for osteoarticular reconstruction
- In vitro seeding of stem cells on these 3D structures and achieving combined implants.
- Achieving some experimental animal models and in vivo application of the therapeutic systems developed.

The entire panel of activities was performed within a complex consortium, formed by organizations with several expertises in the field of cell therapies and technologies orientated towards health. The complementarities between the partners of the consortium must be underlined. The thematic field is oriented towards innovative therapies with potential application in treatment of some chronic disorders, such as osteoarthritis. Age-related degenerative disorders are part of the diseases with a great social and economic impact, requiring hospitalization, rehabilitation procedures and home medical care. Treatment using cell transplant procedures combined with tissue bioengineering techniques will lead to acceleration of functional recovery, shortening of hospitalization period and will decrease the rate of complications.

3. GAIT ANALYSIS USING ZEBRIS MEASUREMENT SYSTEM

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ABSTRACT

The paper presents a study based on experimental measurements developed in order to establish a protocol for human gait investigations. In order to establish which the parameters of interest are, the gait cycles of a number of 20 healthy patients were recorded. The basic concept was to use two different systems in order to achieve the desired information. The main idea was born from the necessity to compare a recording of a healthy person considered as a gait pattern, with a diseased one. The establishing of a normal gait pattern was also a main concern. The combination of the measurement and investigation concepts of the two equipments involved in the study, leads to a protocol for human gait evaluation. In this way, using an investigation protocol and a large database of healthy person recordings, a diagnostic for the diseased persons became facile to establish.

Key words: gait, measurement, investigations, Zebris

4. ENDOTHELIAL DYSFUNCTION AND PULMONARY VASCULAR REMODELING INDUCED BY SMOKING IN PATIENTS WITH COPD

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ABSTRACT
Pulmonary vascular remodeling in COPD patients represents more than a simple hypertrophy of media triggered by long-term hypoxic vasoconstriction. The aim of the present study was to characterize structural changes of pulmonary vasculature in both smokers and patients with mild COPD in order to assess the involvement of cigarette smoking in vascular remodeling initiation. Histological examination of tissue sections dissected from exeresis fragments was followed by the morphometric analysis of muscular pulmonary arteries and a semi-quantitative evaluation of elastin and colagen intimal deposits based on a visual scale. Our results suggest that pulmonary vascular abnormalities present in both smokers and COPD patients with early disease are intimal thickening of small muscular arteries (intimal index was 23.11 ± 3.04 in smokers, respectively 25.62 ± 3.06 in COPD group, v. 17.86 ± 2.96 in control group, $p = 0.005$) and arteriolar muscularisation. In patients with mild COPD, the substrate of intimal thickening is determined initially by the local predominant deposition of elastin and further, by colagen deposition. Immune labeling with anti-CD34 antibodies showed the swelling of endothelial cells as a sign of vascular injury. In conclusion, the direct effect of smoke components on the endothelium may be considered as an important factor responsible for both pulmonary endothelial dysfunction and vascular remodeling.
Key words: intimal thickening, muscular pulmonary arteries, arteriolar muscularisation

5. DOES ALIMENTARY REGIME INFLUENCE SYNOVIAL FLUID IN DOGS WITH ARTICULAR DEFFECTS REPAIRED WITH SCAFFOLDS LOADED WITH CHONDROPROGENITOR CELLS?

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ABSTRACT
Sick or traumatized cartilage has a limited regeneration capacity. Studies show that glucosamine can reduce pain in osteoarthritis and improve articular performances. In the present study we will follow the influence of glucosamine on the inflammatory process and on articular cartilage regeneration in dogs with articular chondral defects surgically induced/treated by synovial fluid analysis.
The study was made on 13 healthy, common breed dogs, in which, under general anesthesia, cartilage defects were made on femoral trochlea and humeral head. The size of the defects was analyzed by CT-

Scan and by 3D reconstruction of obtained data (CEDUCOS / Polytechnic Institute Timisoara), matrix/membranes with size and structure similar with cartilage defects induced were realized and loaded with chondroblasts at Immunology Center of UMF Victor Babes Timisoara. Defects of the humeral head cartilage were filled by injection of 1 cm^3 of chondroblasts suspension. Post-operative, for 5 months the dogs were differentiated feeded in two groups and maintained in the same maintenance conditions. The dogs in experimental group were feeded with special diet, with high glucosamine content (1000mg/Kg). The control group received ordinary dry pet food. Synovial fluid obtained at 5 months post-operative by arthrocentesis after Werner, was examined by refractometry and by microscopic cytology. In both groups, both parameters determined by refractometry (serum proteins and refractive index) were in normal limits, mentioned in literature. In experimental group in which chondroblasts suspension was used, cytology of synovial fluid was in normal limits. The obtained results after joint injection with chondroblasts suspension are similar of those obtained on rabbits. In 80% of individuals in which matrix/membranes, loaded with chondroblasts and fixed with screws were used, an increase of macrophage number and lymphocyte in normal limits were observed. In 80% of individuals from control group fed with normal diet, an increase of macrophages and lymphocyte. Results are similar with those obtained in other studies.

Key words: articular, defects, synovial fluid, refractometry, chondroblasts

6. METHODOLOGICAL OVERVIEW OF CAST 2003 SURVEY

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ABSTRACT

The purpose of this paper is to examine research methods in a survey investigating high school students' behaviour related to alcohol drinking, smoking and illicit drug use. We conducted a cross-sectional study using a stratified cluster sample design. Cronbach's alpha reliability coefficient was determined for the three main parts of the questionnaire investigating alcohol drinking, smoking and drug use. We have observed that design effects tend to be related to the actual prevalence rates of substance use. Thus, rarely used substances such as illicit drugs have low design effects; while more commonly used substances such as cigarettes and alcohol have high design effects. Cluster sampling usually increases the variance of survey estimates. Unequal selection probabilities are accounted for in the analysis of data by computing sample weights for each member of the sample.

Key words: Cronbach's alpha coefficient, design effect, weighting factor

7. INNER EAR VESTIBULAR EPITHELIUM IN MICE CONTAINS PLURIPOTENT STEM CELLS

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ABSTRACT

Recently, scientists discovered that both cochlea and vestibular epithelia in mice, contains stem cells in the first weeks and months after birth. These stem cells have the capacity to differentiate into progenitors of the sensorial cells specific to the organ. Their number is decreasing together with the process of aging. This process seems to explain the loss of the regeneration and proliferation capacity in inner ear sensorial epithelia following different chemical, physical and aging injuries. These were the

motives that have determined us to try to isolate stem cells from vestibular epithelia, to cultivate and finally to differentiate them towards obtaining vestibular and hearing cells.
We harvested utricles from 7 days old mice, which we have tripsinized in order to isolate single cells. Obtained cells were cultivated in thermostat at 37°C and 5% CO2 in DMEM media enriched with F12 Nutrient mixture, B27 andN2 supplement. In order to establish if the cultivated cells were pluripotent, we performed RT PCR to highlight pluripotent markers like Oct 4, Nanog and Gapdh. A specific characteristic of utricular stem cells is the tendency to aggregate and to produce neurospheres in suspension. Our results assure us that utricular epithelia contain a large number of stem cells that can be cultivated and differentiated. The presence of Nanog and Gapdh markers in cultivated neurospheres is a solid argument for their pluripotency.
Isolation, cultivation and in vitro differentiation of vestibular stem cells, where they are more numerous, can become a future modality of realizing regenerative implant in hearing loss.
Key words: cochlea, vestibular epithelium, stem cells, proliferation

8. ABSTRACTS OF THE CONFERENCE REGENERATIVE MEDICINE ERASTEM

HOMING AND ADHESION BEHAVIOUR OF BONE-MARROW DERIVED STEM AND PROGENITOR CELLS

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A regulated adhesion and homing process is the prerequisite for the efficient function of cellular therapies. We have analyzed the homing and adhesion behaviour of hematopoietic stem and progenitor cells (HSCs) in comparison to mesenchymal stem cells (MSCs). MSCs were grown in medium deplete of hematopoietic growth factors, and were $CD45^-$, but $CD73^+$ and $CD105^+$. HSCs were isolated from bone marrow and characterized by lack of lineage specific differentiaton markers (lin^-). Using flow-cytometric analyses, we found that HSCs as well as MSCs expressed the cell surface molecule P-selectin glycoprotein ligand (PSGL)-1. When analyzed in parallel plate flow chambers (PPFCs), both HSCs and MSCs rolled and adhered on immobilized recombinant P-selectin and E-selectin, which are both ligands for PSGL-1, as well as on immobilized endothelial cells. HSCs and MSCs expressed integrins alpha4beta1, alpha5beta1. In contrast to HSCs, we found little expression of beta2 integrins LFA1 and Mac1 on MSCs. The receptor for Stromal Cell Derived Factor (SDF)-1, CXCR4, was expressed on both murine lin^- HSCs and MSCs, and its functionality was confirmed in adhesion assays under shear stress in the PPFC. Human MSCs lacked expression of PSGL-1, but still were able to roll on P-selectin or endothelial cells, indicating that an alternative P-selectin ligand is operative. Intravenous injection of fluorescence-labeled MSCs into mice revealed that both murine and human MSCs distributed between different tissues in a relatively similar way as HSCs, with the exceptions that MSCs (i) failed to accumulate in bone marrow and (ii) showed predominant adhesion in lungs. Taken together, our data show that MSCs are an attractive bone-marrow derived progenitor cell population which maintains substantial part of the homing mechanisms and machinery used by HSCs, and that transplanted culture-expanded MSCs, similarly to HSCs, circulate in blood and home to several tissues.

NK CELLS AND THEIR RECEPTORS

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In this presentation, the immunogenetics of NK cell receptors and their ligands will be reviewed in the context of their expression level related to the KIR and HLA genotypes. At the basis of NK cell biology is the “missing self” concept: NK cells have inhibitory receptors on their surface, which if not triggered lead to lysis of target cells.

There are three types of killer cell immunoglobulin-like receptors (KIR): a) Inhibitory KIR (2DL1,2,3/ 2DL5/ 3DL1/ 3DL2), b) stimulatory KIR (2DS1,2,3,4,5 and 3DS1) and c) KIR2DL4 which has both an activating structure and a cytoplasmic inhibitory motif.
The major KIR specificities are:

KIR2DL1	for	group 2 HLA-C (S77/N80)
KIR2DL/2/3	for	group 1 HLA-C (N77/K80)
KIR3DL1	for	HLA-Bw4 epitope (77-83)

A hallmark of the KIR gene family is their diversity: on the one hand they are highly polymorphic in terms of the presence or absence of genes, leading to highly variable *KIR* haplotypes. On the other hand each of the genes that is present in a given individual is clonally distributed: this term describes the fact that each NK cell clone has a specific combination of KIR that are expressed on the surface. It will be shown that the KIR repertoire results from combinatorial diversity and clonal selection. It depends on the age and the haplotype of the donor. There is a correlation between the KIR expression and the presence of HLA-C ligands in mature but not immature NK cells.
In summary, the detailed analysis of NK cell receptors on the immunogenetic as well as expression level will be necessary to define future strategies, which will exploit the potential of NK cells in autoimmunity, viral infection, and hematopoietic stem cell transplantation.

CELLULAR AND MOLECULAR TARGETED-THERAPY IN MAMMARY CARCINOMA

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Nowadays, mammary cancer is the most frequent malignant neoplasia in females, and the morbidity and specific mortality continue to increase. Many progresses were made in the last years, in the fields of diagnosis and specific therapy, but in terms of epidemiology of the disease, they are far to be enough. This is why basic researches, clinical observations and clinical trials focus on the identification of new targets for adjuvant therapies. The present paper is based exclusively on results obtained in the Laboratory of Molecular Diagnosis of Mammary Carcinoma in the last two years, based on the observations of more than 100 cases. Conventional classification of mammary carcinoma is based on the microscopic observation, by establishing the pathologic form and differentiation. Unfortunately, this classification brings just minor prognostic information, and has no impact on the therapeutic strategy. The recently introduced molecular classification is strongly based on the specific immunophenotype of malignant cells, regarding the expression of cytokeratin 5/6, 18, Her-2/neu protein, c-erbB2, and estrogen receptors. This classification identifies some subtypes of mammary carcinoma, prognosis is significantly more accurate and therapy can be individualized. There are two types of cellular and molecular targets for therapy: accepted, and used as routine procedures (namely estrogen and progesterone receptors, HER-2/neu protein overexpression, and cell proliferation rate, evaluated by Ki67 immunoreaction. Additional targets, some of them found at present time in clinical trials, are E-cadherin, Bcl-2, androgen receptors, prostate specific antigen, anti-angiogenic therapy, and drugs against VEGF/VEGFR system. Their value as diagnostic, prognostic markers, and as targets for specific therapy is presented in relation with the stage and differntiation of the tumor. Future perspectives are mainly related to tumor lymphangiogenesis, which seems to be the key in developing lymph nodes and distant metastasis.

GENETIC ENGINEERING OF CANCER STEM CELLS IN VIEW OF IMMUNE RESPONSE MODULATION IN TUMOURS OF NON-VIRAL ORIGIN

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Background and aims – The concept of cancer stem cell arose from the similarities observed between the self-renewal mechanism of normal organs and continuous proliferation in cancers. Considering the clonal origin of most forms of non-viral tumours, our purpose is to establish a therapeutic strategy to identify, isolate and modify cancer stem cells towards a harmless phenotype.
Methods – We will evaluate the phenotype and characteristic gene mutations of the cancer stem cell population, assessing their differentiation stage and markers involved in neoplastic proliferation and metastatic homing. Based on this analysis, we will atempt to genetically modify the cancer stem cells as to stimmulate the immune response, to induce the differentiation towards a normal, non-tumoral phenotype and to limit their metastatic potential by downregulating the expression of certain chemokine receptors. The immune response will be tested *in vitro*, in mixed cultures, as well as *in vivo* on mouse cancer models.
Estimated results – Our final goal is to design and optimize a vector or genic construct for *in vivo* transfection of cancer stem cells as means of targeted antitumoral therapy, with perspectives of clinical translation.

STEM CELLS AND OSTEOARTICULAR REGENERATION

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Aim of the present study: elaboration and optimisation of procedures for in vitro seeding and culture of mesenchymal stem cells (MSCs) on 3D matrices and evaluation of the in vivo behaviour of those combined implants.
Material and methods: The experiments were performed on 3 groups of animals: group A – articular reconstruction, group B – bone reparation and group C – control group. Each group included 5 healthy dogs. Before the experiments was obtained the approval of Ethics Committees from University of Medicine and Pharmacy Victor Babes Timisoara and from University of Agronomical Sciences and Veterinary Medicine Timisoara. The mesenchymal stem cells were isolated from bone marrow aspirates (harvested by humerus punction). For cell isolation we used the plastic adherence method. The cells were cultivated on semi confluence, trypsinised and seeded in DMEM+10% FCS, 10^5 cells/cm. At second passage the cells were seeded on 3D composite matrices, 10^6 cells/matrix. As scaffolds were tested: plates from anodised titanium alloy, combined or not with hydroxiapatite, silicium plates covered by polyimide, collagen-polyglyicol and collagen-polylactol matrices. Different seeding procedures on 3D matrix were compared as well as conditioning of scaffolds, early or late medium addition. The in vitro study was focused on testing the MSCs adherence and viability on scaffolds. For the in vivo study were surgically induced the bone or joint lesions and the combined implants were placed at the defect site. The dynamic evaluation of healing process was performed by daily clinical examinations, radiological and arthroscopic investigations. At the end of experiments the implants were evaluated by histological sections.
Results and conclusions: The results indicated good cells viability (75-90%) without significant differences between scaffold types, seeding procedure or scaffold conditioning. The MSCs adhered better on collagen based matrices than on the other studied scaffolds. A better adherence was noticed in conditioning scaffolds experiments, without differences regarding the exposure time. Our data suggested that the contact between scaffold and culture media components induced the attachment of cell-cell and cell-matrix interaction factors in a relatively short period of time (2 h). Most of the cells were find at the

border and/or matrix corners, suggesting that the matrix structure can generate geometrical signals for the cell orientation on to the matrices. For the group A, the arthroscopic examinations revealed partially reconstruction of cartilage comparing with the group C which presented practically non-healing process. For the group B the radiological exams confirmed the start of the regeneration process from the defect border which states the new bone synthesis. The histological appearances were consistent with clinical land imagistic findings suggesting that the implant of biomatrices seeded with MSCs may be a valid alternative for the osteoarticular reconstruction.
The study was financed by National Grant CEEX-VIASAN nr. 45/2005

RECONSTRUCTION OF BONE SEGMENTARY DEFECTS THROUGH BIOMIMETIC MATRICES COLONISED WITH OSTEOGENIC CELLS

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Cell therapy sustained by bioactive or biomimetic scaffolds is part of the regenerative medicine which in orthopedics is also called orthobiology. Bone is a natural composite material with 15% of his weight represented by the cell population. In fractures bone has a good regenerative capacity and is one of the few tissues which can heal without a scar. Even so 10% of all fractures evolve to pseudarthrosis from unknown reasons. Also critical bone defects, complication of trauma, sepsis or cancer, which needs surgical large segmental bone resections, can not heal spontaneously and a replacement technique is needed. The bone defect reconstruction is using 10% of the mondial health costs representing a health problem (Yuehuei H. 2006). Due to its intrinsic regenerative capacity bone is an excellent candidate in building reconstructive tissue engineering strategies. Imaging methods to allow segmental bone replacement by ex vivo autologus osteogenic cell cultivation represents a possibility for the autologus organ transplant. Introducing in the therapeutically arsenal of bone regenerative biotechnologies will have a major impact on the health of the target patient. One should not forget that bone trauma and cancer affects the young and very young population.
The first reconstruction of a segmental bone defect of the tibia using bioceramic and stem cells harvested from the patient is presented. The final outcome of the procedure will be the theme of another paper in the future.

INFORMED CONSENT TO COLLECT, STORE AND USE HUMAN BIOLOGICAL MATERIALS FOR RESEARCH PURPOSES. AN INTERNATIONAL FRAMEWORK

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The ethical and legal documents and literature use diverse couples of biobank concepts: *individual* vs. *population* biobanks; *data* vs. *sample* (and data) biobanks; *permanently* vs. *temporarily* biobanks.
The concept of "identifiability" is not uniformly defined. **Identifiable** are considered those biomaterials and data which, alone or in combination with associated data, allow the identification of the person concerned directly or through a code (coded = linked anonymised); **unidentifiable** are considered those biomaterials and data which, alone or in combination with associated data, do not allow, with reasonable efforts, the identification of the person concerned. **Identified** are those biomaterials and data which allow the direct identification of the person concerned through a personal identifier (i.e., name, social security number); **coded (or linked)** are the biomaterials and data which allow the identification of the person concerned through a code; **unlinked (or anonymised)** are the biomaterials and data which,

originally collected in an identifiable way, were subsequently stripped of identifiers or codes allowing the identification of the person concerned; **unidentified (or anonymous)** are those biomaterials and data which lack, by the origin, of personal identifiers.
According to a traditional view of medical and research ethics, the main consent requirements are the **voluntariness** and **information**. To be truly informed, consent should be based on *specific* information on diagnostic or clinical procedures or research programs and the information and consent should refer to *immediate* diagnostic/clinical procedures or research programs.
Conclusions. There is a considerable disharmonisation in regulations, laws and guidelines governing biobanking research; there are a variety of informed consent models for biobanking research; identifiability of human biological materials (hbms) and data impact on the consent requirements; we assist to a difficulty to combine traditional informed consent with consent for biobanking research (prospective biobanking research). There is a pressing need for a harmonisation of regulations on biobanking research and consent requirements. Ethicists and scientists debate how different regulations and consent models could hamper the development of research with hbms, by avoiding the exchange of materials and data. The regulatory and normative differences derive from different ethical frameworks.

IMMUNE-REGULATION OF ALLERGIC RESPONSE: THE IMPORTANCE OF THE BRAKES

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Aims. Th2 cells play a pivotal role in allergen-induced airway inflammation, asthma and allergy. The Th2 cytokines IL-4, IL-5 and IL-13, indeed, promote IgE production, eosinophil differentiation and airways hyperreactivity, respectively. GATA-3 and T-bet are nuclear factors that promote epigenetic modifications required for Th2 and Th1 cell differentiation, respectively. CTLA-4 is a receptor that critically regulates T cell activation and differentiation. In humans, CTLA-4 polymorphisms are associated with increased IgE serum concentrations and allergies. The aim of our studies is to investigate the role of CTLA-4 in Th2 cell differentiation and IgE production.
Materials/methods. *Naïve* CD4 cells were stimulated *in vitro* under Th1-, Th2-polarizing and neutral conditions. For *in vivo* experiments, mice were immunized with the allergen Parj1 and treated with anti-CTLA-4 mAb. Cytokine production and Ig serum levels were analysed by ELISA; T-bet and GATA-3 mRNA expression by RT and real time PCR; STAT-6 phosphorylation by cell-based ELISA; IL-4R□ chain by western blot; cells expressing IFN-□, IL-4 and GATA-3 proteins by flow cytometry.
Results. CTLA-4 stimulation inhibits Th2 but not Th1 cell differentiation. GATA-3 mRNA expression is inhibited by CTLA-4 when CD4 cells are stimulated *in vitro* under both neutral and Th2-polarising conditions. CTLA-4 stimulation also inhibits STAT6 activation and IL-4Rα up-regulation. *In vivo* CTLA-4 functional blockade skews Ig production towards IL-4-dependent Ig isotypes, the serum levels of Parj1-specific antibodies being increased for IgE and decreased for IgG2a. Consistently, CTLA-4 blockade increases the frequency of Parj1-specific Th2 cells but not Th1 cells as well as IL-4 and IL-5 but not IFN-γ production. Moreover, CTLA-4 blockade enhances GATA-3 protein level per cell.
In **conclusion**, CTLA-4, by affecting the level of GATA-3/cell, contributes to keep this factor under the threshold required to become a Th2 effector cell. Consequently, it affects IgE/IgG2a production and contributes to the outcome of allergen-specific immune responses.

CELL THERAPY: USE OF STEM CELLS IN ANIMAL MODELS

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In Stefan S. Nicolau Institue of Virology, Bucharest, Romania, we develop studies of molecular mechanisms which sustain the ex vivo expansion of the adult stem cell (hematopoietic, mesenchymal, hepatic) in order to find effective methods, technically relevant, for ex vivo cultivation of these cells, through manipulation of culture microenvironment by cytokines supplementation. The general objective is to bring stem cell technology to the clinic and to initiate cell therapy procedures in patients with malignant disorders, hepatic failure and inborn congenital disorders.

The aim of one project (CEEX 85/2006) is to expand *ex vivo* the primitive compartment of multipotent hematopoietic stem cells and to induce differentiation in order to obtain a cell population able to rapidly sustain hematopoietic recovery in myelosuppressed mice.

Material and methods. The study was designed to evaluate several culture systems for *in vitro* maintenance of umbilical cord blood stem cells. The influences of different growth conditions have been evaluated on CD34+ cell expansion, immunophenotyping, cell cycling and maintenance of clonogenic progenitors. For cell expansion we used murine stromal feeder layer, various cytokines cocktails and placental conditioned medium, a feeder layer-free culture system that allows elimination of the xeno-components.

Results. The effect of different protocols on the expansion of CD34 cells revealed the significant impact obtained by addition of Tpo, SCF, Flt3 ligand and IL6 comparing with other growth factor combinations.

Conclusion. The results of our study clearly show that the *ex vivo* expansion of hematopoietic progenitor cells obtained from human umbilical cord blood is dependent on controlled experimental conditions, which might be helpful when designing culture systems for clinical applications.

The second project goal (CEEX 139/2006) is to test the role of transplanted mesenchymal stem cells (MSCs) in bowel wall repair, to obtain a nontoxic, nonallergenic and biological resistant patch.

Mesenchymal stem cells MSCs determine an increasing interest due to their therapeutic value. MSCs seem to play a significant role in adult solid-organ tissue repair.

Materials and methods. We used biosynthetic 3D patches with MSCs to substitute a portion from the rat bowel wall. We obtained MSCs from rat, pig and human and we cultured them in vitro. In order to obtain the 3D patches we seeded these cells into a collagen-agarose polymer. For reconstruction of the bowel wall we sutured the 3D patches using microsurgery techniques.

Results. The polymeric compound was analyzed with electronic microscopy and the cultivated MSCs were analyzed with flow cytometry. The 3D patches were tested for cellular toxicity. The rats were sacrificed at different period of time postoperative and the patches were histologically and immunohistochemically analyzed. It was observed that our 3D patches contributed to the regeneration of the four layer of the bowel wall.

Conclusions. Our study describes the obtaining, the isolation, the culture and characterization of the MSCs in order to use them for the repair of bowel wall defects. Our study demonstrated that 3D patches with MSCs play an important role in repair of the bowel wall defects. We intend to further test these 3D patches in an animal intestinal fistula model.

Other project aim is to develop a new promising therapeutical approach in liver stem disease (CEEX 65/2006). As the disparity between patients waiting for liver transplantation and available organs grows, there are increasing efforts to find potentially viable alternatives, such as adult living donor liver transplantation, use of marginal donors (older donors, diabetics, steatotic grafts), use of ABO incompatible or non-heart-beating donor, but also transplantation of mature hepatocytes or of stem/progenitor cells and potential of transplanting xenogeneic organs and cells. The adult stem cells from mobilized peripheral blood may represent a considerable advantage in their use for the therapy of hepatic lesions because could be harvested from live donors by a minimally invasive technique. The proposed research has the potential to increase the data about liver stem cell biology, representing a new source of data for the future clinical trials. By developing a new cellular therapy in liver disease we will increase the therapeutical approach in this field.

INDOMETHACIN INHIBITS THYMIC INVOLUTION IN MICE WITH STREPTOZOTOCIN-INDUCEDDIABETES

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Diabetes is a chronic disease that is accompanied by a rapid thymus involution. To investigate the factors responsible for thymic involution in a model of STZ-induced diabetes, mice were injected with STZ alone or in combination with the cyclooxygenase 2 inhibitor indomethacin (INDO). Thymus weight, glycemia and serum corticosterone were measured, and apoptosis in thymus and thymocyte cultures was analyzed by flow cytometry. Although earlier studies report that streptozotocin (STZ) is toxic to lymphoid tissues, in our experiments even massive doses of STZ did not negatively affect thymocyte cultures. Cultured thymocytes also seemed unaffected by high glucose concentrations, even after 24 h of exposure. Administration of INDO concomitantly with STZ reduced thymic involution but did not prevent the onset of hyperglycemia or reduce established hyperglycemia. When INDO was given before STZ, the same degree of thymic involution occurred; however, hyperglycemia was reduced, although normoglycemia was not restored. INDO also reduced serum corticosterone. Because thymocytes are known to be sensitive to glucocorticoids, this finding suggests that cyclooxygenase 2 inhibition may retard thymic involution by reducing serum glucocorticoids. In conclusion, our results show that STZ and hyperglycemia are not toxic to thymocytes and that cyclooxygenase-2-mediated mechanisms are involved in thymic involution during diabetes.

HUMAN MESENCHYMAL STEM CELLS TROPISM FOR GLIOBLASTOMA XENOGRAFT IMPLANTS IN NUDE MICE: EXPERIMENTAL MODELS

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Introduction: The researches of the last years evidenced an extremely interesting property of the neural stem cells, namely their tropism towards the cerebral malign tumor cells (Abody and collab., 2000). Recent researches revealed also the capability of the stem cells extracted from the bone marrow to “trace” the cerebral tumor malign cells along their route of metastatic insemination, showing the same tropism for the tumor malign cells as the neural stem cells (Nakamizo and collab., 2005).
Purpose: The purpose of this study is to assess the tropism of human mesenchymal stem cells (hMSC) for glioblastoma (GBM) cells for the perspective of using them as cellular vectors for tumoricide genes (IL-2, TNF-alpha, TRAIL, etc) as a potential therapy for glioblastoma.
Material and methods: We used several groups of nude mice in order to asses the tropism of hMSC for GBM cells (U87 MG). The U 87 cells marked with green fluorescent protein gene (GFP) were inoculated intra-cerebral. After 7 days we inoculated in the opposite cerebral hemisphere, contra laterally, the labeled hMSC. In the control group we inoculated phosphate buffered solution (PBS) intra-cerebral and after 7 days labeled hMSC in the opposite cerebral hemisphere. At different times the samples were obtained, processed and studied using immunohistochemistry/ immunofluorescence techniques.
Results: The tropism of hMSC for U87 cells was assessed by comparing the labeled hMSC at the level of the inoculation hMSC site and around the U87 GFP marked cells in the contralateral hemisphere, at 14 days after hMSC inoculations. We use the control group to see if the migration of hMSC is due to the inflammatory process which appears at the inoculation site or is the results of the presence of the glioblastoma cells.
Conclusions: The confirmation of hMSC tropism for glioblastoma cells it is important for the perspective of clinical application, because mesenchymal stem cells are easy to be obtained from the

patient during the surgical procedure. These cells could be transfected with one of the tumoricide gene (IL1, TRAIL, TNF,etc.)
Then the transfected hMSC could be inoculated by one of the following routes: intracerebral (during the re-intervention procedure) or intra-carotid by an angiographic procedure.

GAIT ANALYSIS USING ZEBRIS MEASUREMENT SYSTEM

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Gait analysis is used to discover and evaluate abnormal movements, which are connected with different diseases in joints, muscles, etc. Based on laboratory measurements of gait analysis, in correlation with a consultation, doctors are able to diagnose different disorders or monitor the patient rehabilitation.
The paper presents a study based on experimental measurements developed in order to establish a protocol for human gait investigations. In order to establish which the parameters of interest are, the gait cycles of a number of 20 healthy patients were recorded. The basic concept was to use two different systems in order to achieve the desired information. The main idea was born from the necessity to compare a recording of a healthy person considered as a gait pattern, with a diseased one. The establishing of a normal gait pattern was also a main concern.
The recording equipments used for experimental measurements are: the human gait analysis system Zebris CMS-HS, and the plantar force measurement system Zebris FDM.
The Zebris measuring system for gait analysis enables simple and fast analysis of all important parameters of the human gait. The investigations using Zebris CMS-HS system leads to a kinematical approach by analyzing the angular variation of each joint. The Zebris FDM measuring system functions using high-quality capacitive force sensors that are arranged in matrix form. The measuring plate enables both the static and dynamic plantar force distribution to be analyzed during the patient standing and walking. The measurements are processed on the computer using the WinFDM software package.
The combination of the measurement and investigation concepts of the two equipments involved in the study, leads to a protocol for human gait evaluation. In this way, using an investigation protocol and a large database of healthy person recordings, a diagnostic for the diseased persons became facile to establish.

THE TiO_2-Pt NANOPARTICLES INTERACTION WITH ANIMAL CELL AND THEIR UTILIZATION IN MEDICINE

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Initially considered inert from the biological point of view, recent researches point out that the ultrafine particles of TiO_2 (under 30 nm); can induce some cytotoxic effects, especially in lung, in experiment on rats. In this work titanium dioxide was doped with platinum ions and it was synthesized by sol gel method. The precursor for titanium was titanium tetrachloride, and for platinum it was used acid hexachloroplatinate hydrate (1% Pt form titanium quantity). The obtained material was characterized through X-ray diffraction (XRD) and scanning electron microscopy (SEM). From diffractogram analysis, it results that the *anatase* crystallization form was obtained.
TiO_2-Pt nanoparticles were intraperitoneal injected in *Mus musculus*, exposed or not at a stress factor (X-rays). The TiO_2-Pt nanoparticles stimulated the immune system, conducting to the enhanced of the immunoglobines quantity (IgG, IgM) confirmed by the hemoleucogram values. The amount of IgM and IgA recorded similar values at all variants (irradiated or not), injected with TiO_2-Pt nanoparticles, these stimulating the immune response of the animals. At the hepatic liver level, the TiO_2-Pt nanoparticles were presented in the sinusoid capillaries (circulatory system) and in the Kupffer cells (especially in

endoplasmic reticulum, cytoplasm and in vacuoles). Under the action of X-rays, the hepatocyte ultrastructure at mouse was altered, being affected especially the nucleus, mitochondria and endoplasmic reticle. Also it was affected the quantity of drops lipid and glycogen amount from the cell, as well as the Kupffer cell ultrastructure.
The nanoparticles are active eliminated at the un-irradiated animals. In irradiated animals, in some Kupffer cells there are not present the TiO_2-Pt nanoparticles, which remain in the circulatory system, activating the immune system. The TiO_2-Pt nanoparticles presence in organism, determined an intensification of the cellular metabolism, in hepatocytes being lipid drops, and nucleus and cellular organelles having a normal structure.

FUNCTIONAL GENOMICS STUDIES IN CANCER BIOLOGY

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Introduction: The main purpose of functional genomics studies was to identify and validate some new molecular biomarker involved in tumor angiogenesis through different functional genomics tools. We evaluated the ovarian cancer progression looking for the two important steps invasion and metastasis. We performed protein array, microarray and RNA interference for the validation of angiomarkers in ovarian cancer.
Material and methods: The study was divided in two major areas: *in vitro* on cell culture for RNA interference and RT-PCR and on clinical samples from patients with ovarian cancer stored in liquid nitrogen for microarray and Fast Quant array. We used the Agilent microarray technology for gene detection and siRNA for inhibition of mRNA expression on several RNAs: VEGF, VEGFR, TNF-α, MMP-9. The RT-PCR was performed on tumor cell culture for the same biomarkers.
Discussion and conclusions: Our results suggest that that for biomarker validation there are necessary several types of analysis as the array for gene expression or serum as well as the control used for cell culture. The advantage of all of the above is the high quality analysis and the reproducibility of the results. The capacity to define and validate the angiogenesis biomarkers is given by the high potential of the tools performed in the study.

DOES ALIMENTARY REGIME INFLUENCE SYNOVIAL FLUID IN DOGS WITH ARTICULAR DEFFECTS REPAIRED WITH SCAFFOLDS LOADED WITH CONDROPROGENITOR CELLS?

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Sick or traumatized cartilage has a limited regeneration capacity. Studies show that glucosamine can reduce pain in osteoarthritis and improve articular performances. In the present study we will follow the influence of glucosamine on the inflammatory process and on articular cartilage regeneration in dogs with articular chondral defects surgically induced/treated by synovial fluid analysis.
The study was made on 13 healthy, common breed dogs, in which, under general anesthesia, cartilage defects were made on femoral trochlea and humeral head. The size of the defects was analyzed by CT-Scan and by 3D reconstruction of obtained data (CEDUCOS / Polytechnic Institute Timisoara), matrix/membranes with size and structure similar with cartilage defects induced were realized and

loaded with chondroblasts at Immunology Center of UMF Victor Babes Timisoara. Defects of the humeral head cartilage were filled by injection of 1 cm^3 of chondroblasts suspension.
Post-operative, for 5 months the dogs were differentiated fed in two groups and maintained in the same maintenance conditions. The dogs in experimental group were fed with special diet, with high glucosamine content (1000mg/Kg). The control group received ordinary dry pet food.
Synovial fluid obtained at 5 months post-operative by arthrocentesis after Werner, was examined by refractometry and by microscopic cytology.
In both groups, both parameters determined by refractometry (serum proteins and refractive index) were in normal limits, mentioned in literature.
In experimental group in which chondroblasts suspension was used, cytology of synovial fluid was in normal limits. The obtained results after joint injection with chondroblasts suspension are similar of those obtained on rabbits.
In 80% of individuals in which matrix/membranes, loaded with chondroblasts and fixed with screws were used, an increase of macrophage number and lymphocyte in normal limits were observed. In 80% of individuals from control group fed with normal diet, an increase of macrophages and lymphocyte. Results are similar with those obtained in other studies.

Conclusions

1. The increase of lymphocyte and macrophage number in individuals fed with normal diet, denote presence of degenerative modifications developed secondary to articular defects induced by surgery;
2. Incomplete cover or uncover of screw head by new born articular cartilage determined the maintain of an intraarticular irritation which does not permit the evaluation of the glucosamine implication in the healing process of induced articular cartilage defects;
3. Increase of macrophage number and normal levels of lymphocyte in individuals with matrix/membranes fixed with screws; denote the presence of immunologic nonspecific reaction (foreign body reaction).

ENDOTHELIAL DYSFUNCTION AND PULMONARY VASCULAR REMODELING INDUCED BY SMOKING IN PATIENTS WITH COPD

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Pulmonary vascular remodeling in COPD patients represents more than a simple hypertrophy of media triggered by long-term hypoxic vasoconstriction. The aim of the present study was to characterize structural changes of pulmonary vasculature in both smokers and patients with mild COPD in order to assess the involvement of cigarette smoking in vascular remodeling initiation. Histological examination of tissue sections dissected from exeresis fragments was followed by the morphometric analysis of muscular pulmonary arteries and a semi-quantitative evaluation of elastin and collagen intimal deposits based on a visual scale. Our results suggest that pulmonary vascular abnormalities present in both smokers and COPD patients with early disease are intimal thickening of small muscular arteries (intimal index was 23.11 ± 3.04 in smokers, respectively 25.62 ± 3.06 in COPD group, v. 17.86 ± 2.96 in control group, $p = 0.005$) and arteriolar muscularisation. In patients with mild COPD, the substrate of intimal thickening is determined initially by the local predominant deposition of elastin and further, by collagen deposition. Immune labeling with anti-CD34 antibodies showed the swelling of endothelial cells as a sign of vascular injury. In conclusion, the direct effect of smoke components on the endothelium may be considered as an important factor responsible for both pulmonary endothelial dysfunction and vascular remodeling.

HUMAN ADULT STEM CELLS INVOLVEMENT IN CARDIOVASCULAR TISSUE ENGINEERING

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Introduction. The objectives of our study were:
1. HSC isolation and culture from different sources (bone marrow, peripheral blood, umbilical cord and placenta blood) for endothelial lineage differentiation;
2. To assess the safety and feasibility of bone marrow derived autologous cell transplant, using intra-coronary infusion at the level of infarcted artery, in patients suffering from recent and acute myocardial infarction.

Material and methods: HSC isolation was performed by positive selection using electromagnetic beads directly conjugated with CD34 monoclonal antibodies (for peripheral and umbilical cord and placenta blood) and AC133 (for bone marrow). Cells were cultured in special medium with growth factors. HSC differentiation towards the endothelial lineage was performed by adding cytokines, the essential ones being VEGF, SCGF and ECGF. We elaborated a protocol for intra coronary infusion of these cells to patients with acute myocardial infarction.

Results: In order to separate HSC we used complete separation kits from DYNAL Biotech and Miltenyi Biotec (MACS), and we preferred the last one due to its advantages revealed during the experimental procedures. HSC differentiation towards the endothelial lineage was performed by adding cytokines, the essential ones being VEGF, SCGF, and into a lesser extent ECGS, which was used only during the experiments performed on HSC isolated from cord blood and placental blood. Presence of VEGF in a relatively high concentration (50 ng/ml) was absolutely necessary for differentiation towards the endothelial lineage of HSC isolated from peripheral blood and bone marrow. Both bone marrow, as well as the peripheral blood can be considered as reliable sources of endothelial cells, which can be used for stent endothelization. Cord blood and placental blood contain a relatively small amount of HSC (0.1-0.5%), but their multipotent capacity proved to be significantly increased compared to peripheral blood HSC, this blood is the easiest to harvest, being considered a waste product.
After 9 months, segmentary perfusion was improved for all patients with acute myocardial infarction.

Conclusions: Results of our study showed that it is possible to obtain endothelial cells from HSC. Stem cell therapy is a new approach for the management of myocardial diseases. Designing clinical trials that allow us to adequately evaluate the safety and efficacy of cell transplantation is mandatory.

ROLE OF 5-AZACYTIDINE IN THE TRANSFORMATION OF MESENCHYMAL STEM CELLS IN CARDIOMYOCYTES

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Obtaining muscular cells by differentiation from adult mesenchymal stem cells is a method of great interest. The stem cells were obtained from fresh bone marrow on a separation gradient. Under stimulation with 5- azacytidine (5-aza) within one week about 30% of the stem cells underwent the desired morphological changes. In a few areas connections between cells occurred in a tissue like formation. After four weeks of 5-aza stimulation the cell density started to decrease especially in the continuous stimulated cultures. The expression of Myogenin and MYF5 was assessed by RNA quantization. The expression of the two markers was significant in the stimulated cultures. Continuous culture induces higher gene expression in the presence of 5-aza while the intermittent stimulation produces a better cell survival.

DIFFERENTIATION OF MESENCHYMAL STEM CELLS TOWARDS DIFFERENT CELL LINEAGES UNDER THE INFLUENCE OF CULTURE MEDIUM FACTORS

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In order to underline the pluripotent capacity of mesenchymal stem cells, differentiation procedures were performed towards the 3 usual cellular lineages (osteoblastic, chondrocytic and muscular). *Osteoblastic differentiation* involved isolation, culture until reaching 60-80% confluence, trypsinization and re-plating in DMEM + 10% FCS (10^5cells/cm^2 density). At the second passage, the cells were in vitro seeded on 3D scaffolds (10^6cells/matrix density). Dexamethasone, ascorbate-2-phosphate and β-glycerolphosphate were added in culture medium and cellular viability was assessed using Trypan Blue staining. After 14 days, bone mineralization was tested using von Kossa reaction. For *chondrocytic differentiation*, isolated MSC were cultured, and when reaching semi-confluence (3 weeks) the cells were passed. After the second passage, the cells were placed in medium for induction of chondrocytic differentiation, using BMP2 or TGF beta as inductor agents. Two culture methods were used – in monolayer suspension or micro-aggregates. Cells were analyzed and characterized using light microscopy, immunohistochemistry, flowcytometry and molecular biology (RT-PCR). *Muscular differentiation* used isolated MSC cultured in usual medium to which 5-azacytidine was added for induction of differentiation (continuous and discontinuous). RNA extraction was then performed using TRIzol kit, reverse transcription and quantitative PCR (Taq/Man amplification), in order to assess expression of genes encoding the myogenin.

P63 AND THE EPITHELIAL STEM CELL: VALUE OF mRNA LEVEL IN CERVICAL SMEAR

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Uterine cervix is covered with three epithelial types: cylindrical, squamous and metaplasic, exhibiting different vulnerability to neoplasic processes. Epithelial turnover in this area are thought to be different from neighboring stabile structure. One multipotent cell from this area has the ability to differentiate as cylindrical cells and squamous cells, ensuring the cover of nude surfaces at the transformation zone level. Probably, this is the mechanism for local epithelial regeneration after pregnancy. Along with this process, like embryonic development, anomalies that uncouple proliferation from differentiation generating cellular clone with high multiplication potential can appear. A lot of question remains unanswered till now: (i) which is the origin of these cells and (ii) are they all time in the epithelial structure or appear only in some circumstances?

On the other hand, cervix anatomy is such that early detection of a disease with a Pap smear can predispose early intervention of HPV in tumor progression. Several studies showed correlation between papillomavirus life cycle and differentiation program of infected host cell, in order to produce mature virions in squamous, differentiated cells. Viral DNA is maintaining at low copies number in nucleus of infected cells that are subjected to differentiation and are pushed out of the surface epithelium. The majority of cervical HPV infections are transient non-neoplastic productive viral infections which disappear within months. By contrast, persistent HPV infections are much more likely to progress to pre-malignant and malignant cervical lesions than transient productive infections. The crucial factors for persistence of HPV infections in women with normal immune status are still unknown. In these conditions, is thought that viral persistence only occurs upon targeted infection of specific cervical cells such as stem cells.

Extension of stem cell theory at solid tumor offers support for new hypothesis in tumorigenesis. This important but in the same time so discreet cell is intensively studied in order to associate malign behaviors with specific niche alteration. In this context, we studied p63 expression as epithelial marker

to distinguish between normal and transformed profile. It was admitted that p63, a homologue of the tumor suppressor p53, is critical for the development and maintenance of squamous epithelia. p63 is specifically expressed in the basal layers of stratified epithelial tissues being considered a specific marker, but the role of p63 in tumorigenesis remains poorly defined.
In order to understand the mechanism that governs balance between epithelial regeneration and transformation we investigated p63 expression in imunohistochemistry. To elude this invasive method we quantified p63 mRNA levels (like marker that is critical for controlling epidermal morphogenesis) in smear of women with or without positive test for HPV. p63 expression was identified in basal/subcolumnar cells. Metaplastic (squamous or mucinous) epithelia, either alone or in conjunction with hyperplasias or carcinomas, exhibited the most intense staining, primarily in basal or subcolumnar cells. Dysplasia was characterized by exceeding range of 1/3 epithelial immunostaining. mRNA p63 levels were growing up in association with high risk HPV presence and stage of disease.
Probably, the over-expression of p63 (a molecular shifter of epithelial stratification) leads to increased oncogenic activity in association with HPV (a selective pressure during tumorigenesis).

ETHICAL TRENDS IN MEDICAL RESEARCH

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Aim: this paper intend to present starting from the general ethical principles to the actual problems raised in academic area on the charge of ethics committees.
Discussions: Knowing the four general principles of bioethics presently allows variations which are useful in research. The application of these principles proves that they cannot be separated and on the contrary they interact with one another. Thus the continuous respect for the principle of autonomy and that of justice has been supplemented starting with the Nuremberg code, then with the first declaration from Helsinki (1964) which was recently modified, in 2004, when it was established that the right of the subjects to join in the benefits of the medical research must be clearly stipulated in the project. Regarding the respect for human dignity, deemed as an intrinsic value of the human being, or example, the selection of the receiver of a transplant on strictly medical criteria is provided. The procedures by which one pursues the compliance with the ethical regulations of research have lead to the founding of ethical committees. At the moment, both at a national level and at the level of the medical universities and medical units, such committees have been founded. These independent organisms comprised of famed persons belonging to both medical and non-medical fields, with the responsibility of assuring the protection of the rights, safety and wellbeing of the subjects implicated in a study, but also of ensuring public control of these protections as it is stipulated in the 2001/20/EC Directive of the European Parliament.
Conclusions: medical universities must be focused on ethical education of their students and in supervision of every research project.

AFM METHODOLOGY TO EVALUATE THE VISCOELASTIC PROPERTIES OF CARTILAGE

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Atomic force microscopy is now a known technique for investigating different biological systems at nanometer scale. This allows as imaging in the native state, but also can be used to reveal intrinsic mechanical properties like stiffness or flexibility or to characterize the surface. All that data can be acquired using Nanoscope by Veeco. This microscope has the potential to image biological preparations in real time under nearby physiological conditions with nanometer resolution.
The unit is designed for imaging small -1.5 cm diameter - sample and is able to provide images from nanometric scale to 175μm in size. Typically, samples are fixed on metal disks and then attached to the

top of the scanner tube. The scanner moves back and forth with the sample on allowing to the cantilever to extract information from the surface. This are transmitted to the computer. This work in 2 ways: Real-Time and Off-Line.

The Real-Time software is running the microscope, changing the size and location of scanning, etc. Then images obtained can be analyzed or modified by Off-Line functions, correcting the noise and artifacts, analyze for depth, roughness, etc. A major advantage is that both modes can be run simultaneously, because it is possible to save images in Real-Time mode while the operator is analyzing earlier images in Off-Line mode.

There are two techniques that are most used on biological samples: Contact Mode AFM and Tapping Mode AFM and both can operate in air or fluids. Usually, Contact Mode is for more rough samples, with changes in topography, like collagen or cartilage, d for more smooth samples like cells it is used Tapping Mode. So, for choosing the technique it is important to know very well the sample.

Contact Mode AFM operates by scanning a tip attached to the end of a cantilever across the sample surface while monitoring the change in cantilever deflection. The properties and dimensions of the cantilever play an important ole in determining the sensitivity and resolution of the AFM. For Contact Mode it is necessary to have a soft enough cantilever to be deflected by small forces and with high resonant frequency not to be susceptible to instabilities. These are accomplished by Silicon Nitride probes. There are 4 cantilevers with 4 different springs constant. The spring constant represents the amount of force that makes the cantilever to band during the working. Although they have a nominally value from the manufacturer, there are differences that are important in calculating the Young Modulus of the sample. For the measurements to be accurate, the same tip with the same cantilever spring constant must be used. So it is important to choose proper and good cantilever and than to calculate its spring constant. For this probe the cantilever configuration is V-shaped. They are 100 and 200 micron long, the 2 wider are on top and 2 slimmer are on the bottom. The most used is the slimmer one with 200um and a spring constant of 0.06 N/m.

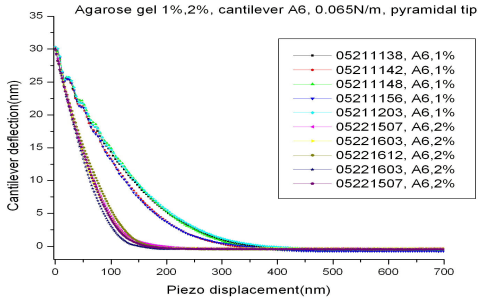
Force plots are used to measure tip sample interactions and to determine the optimal set-points. The second step is sensitivity determination. This represents the cantilever deflection signal versus voltage applied to the piezo and must be done in order to achieve accurate deflection data. Sensitivity is equal to the slope of the force curve while the cantilever is in contact with the sample surface. This involve the following steps: obtain a good force curve on the display monitor, position the cursor on one end of the contact portion of the curve, click with the left mouse to fix the line segment, drag the mouse to position the line parallel to the contact portion of the force curve, the second click makes the system to calculate the slope and enter this value as Sensitivity in the menu.

The third step is to find good curves and to interpret Force Curve. An examination of force curves can be useful in determining the characteristics of the samples: adhesion, stiffness and the repulsion-attraction force between tip and samples.

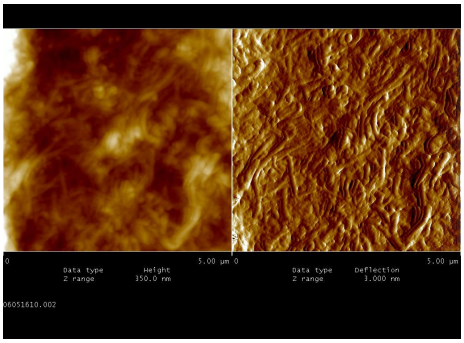
The fourth step is to extract the curve and to calculate the Young Modulus. To derive as much reliable information from the load displacement curves as possible, the data from upper 75% of the unloading curves are used because they are noise free.

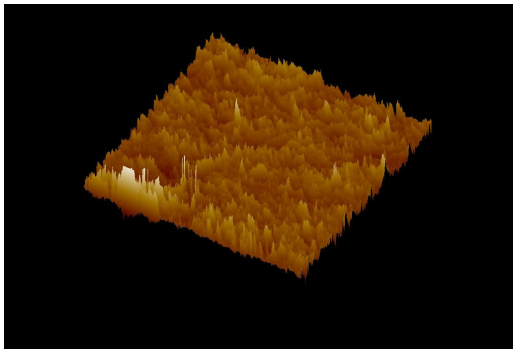
Agarose gels can be used as a tissue-like material because represent a high water content organic material, can be prepared in a standard manner and can be studied for mechanical properties both in nanometer and micrometer scales by indentation testes and force imaging. This is a simple and reliable model for the reference curves.

In our work, we can use different concentration of the agarose gels: 1%, 2%, 2.5%. The goal is to obtain the right curves, with statistically difference between them using the same parameter set.



The cartilage is a visco-elastic material because of its structure and the ability to move water during the movements. Using the AFM we can obtain image, but we can also describe the mechanical properties. It was described that in normal joint cartilage the collagen fiber are random distributed – like in the next image, with a tendency to parallel fiber in ageing cartilage.





INNER EAR VESTIBULAR EPITHELIUM IN MICE CONTAINS PLURIPOTENT STEM CELLS

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Introduction: Recently, scientists discovered that both cochlea and vestibular epithelia in mice, contains stem cells in the first weeks and months after birth. These stem cells have the capacity to differentiate into progenitors of the sensorial cells specific to the organ. Their number is decreasing together with the process of aging. This process seems to explain the loss of the regeneration and proliferation capacity in inner ear sensorial epithelia following different chemical, physical and aging injuries.
Aim of work: These were the motives that have determined us to try to isolate stem cells from vestibular epithelia, to cultivate and finally to differentiate them towards obtaining vestibular and hearing cells.
Matherial and methods: We harvested utricles from 7 days old mice, which we have tripsinized in order to isolate single cells. Obtained cells were cultivated in thermostat at 37°C and 5% CO2 in DMEM media enriched with F12 Nutrient mixture, B27 andN2 supplement. In order to establish if the cultivated cells were pluripotent, we performed RT PCR to highlight pluripotent markers like Oct 4, Nanog and Gapdh.
Results: A specific characteristic of utricular stem cells is the tendency to aggregate and to produce neurospheres in suspension. Our results assure us that utricular epithelia contain a large number of stem cells that can be cultivated and differentiated. The presence of Nanog and Gapdh markers in cultivated neurospheres is a solid argument for their pluripotency.
Conclusions: Isolation, cultivation and in vitro differentiation of vestibular stem cells, where they are more numerous, can become a future modality of realizing regenerative implant in hearing loss.

PRACTICAL ASPECTS OF IMMUNOHISTOCHEMISTRY TECHNIQUE

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Immunohistochemistry (IHC) is a widely spread technique which allow visualization of antigens expression at the tissue level, on microscopic preparation. This presentation is reviewing the principles of this method, the working protocols, as well as the main problems that can occur, together with their possible solutions. Different classes of antibodies and their characteristics are also presented; technical variants of performing the reaction; methods of antigenic revealing and the most encountered situations in medical practice; methods of providing quality control. All data are based on field experience of Pathologic Anatomy Service of Bucharest Universitary Emergency Hospital.

SENTAP:THE IMPACT OF IMMUNOSENESCENCE

Conf. Univ. Dr. Med. Daniel P. Cioca, Dr. Daciana Rusu, Dr. Elena Dinca, Biol. Simona Anghel

Immunogerontology is a novel domain at the interface between immunology and gerontology, a multidisciplinary science which deals with the biological, social, economical, psichological and health aspects of the aging process. Immunogerontology has as a main objective the study of different aspects of the immune system functionality in elderly people, and also the immune disfunctionalities characteristic for this age. Nowadays there is a growing number of scientifical data which point to the fact that the immune system's decay in functionality with aging, a process named immunosenescence, is substantially contributing to the elderly's morbidity and mortality by increasing the incidence of infections and by the increase frequency of neoplasic diseases in the elder people.
The purpose of this study was to assess the major changes in the immune system's functionality in the elderly. Aging is accompanied by a shift in CD8 T cell differentiation manifested by low naïve and high memory / effector T cell counts, effector cell accumulation being accelerated in persons with latent CMV infection. Of special interest was the distribution of surface CD5 and its modulation in T cell subsets of young and elderly people. CD5 is a negative regulator of T cell receptor signaling, and its incr eased expresion on T cells from elderly people is imposing a higher activation threshold, resulting in lower proliferation rates and apoptosis resistance.