Abstracts: XXXVI Annual ESAO Congress, 2-5 September 2009, Compiègne - France

01 EXTENDED IN-VIVO EVALUATION OF A MINIATURIZED LVAD FOR MINIMAL INVASIVE IMPLANTATION
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Objectives: To minimize surgical trauma within the implantation of LVADs, minimally invasive techniques are pursued. We developed such an approach using a micro-axial pump positioned in the right chest cavity and with inflow cannulation via a pulmonary vein entering into the left ventricle. The system was evaluated in initial 30-day implementations in sheep.

Methods: A Heartware HVAD microaxial pump (length 55mm, diameter 21/31mm) with a maximum available flow of 6 L/min was combined with a recently developed inflow cannula with a new flow-optimized tip, and an outflow graft to the ascending aorta. The inflow was implanted into 8 sheep (70±10 kg). The cannula was inserted via the superior pulmonary vein passing through the left atrium into the left ventricle. No anticoagulants or antiplatelet drugs were administered after surgery. The implants were retrieved 30 days following implantation.

Results: Sheep finished the 30-day investigation period. One animal was electively terminated after a controller failure at day 17, and one due to a leak caused by prototype mounting. In the last series of 3 sheep peak flows between 6.1 and 6.9 L/min could be achieved. Mean support flow was set to about 3L/min. Neither signs of mitral valve lesions nor thrombus formation around the cannula, the tip and particularly the insertion site were observed. Except for a myocardial infarction in one animal no signs of thromboembolic events were detected.

Conclusions: The excellent results of these initial implantations demonstrate the feasibility of the implantation and cannulation technique, the proper design of the cannula tip and the capability to provide high flow support.

02 AN OVINE MODEL OF POSTINFARCTION REMODELLING
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Objectives: Though the burden of Ventricular Assist Device (VAD)-recipients suffering from ischemic cardiomyopathy, reverse remodelling by mechanical unloading has been poorly studied in this patient population. We therefore suffering from ischemic cardiomyopathy, reverse remodelling by mechanical unloading has been poorly studied in this patient population. We therefore

Methods: A HeartWare LVAD microaxial pump (length 55mm, diameter 21/31mm) with a maximum available flow of 6 L/min was combined with a recently developed inflow cannula with a new flow-optimized tip, and an outflow graft to the ascending aorta. The inflow was implanted into 8 sheep (70±10 kg). The cannula was inserted via the superior pulmonary vein passing through the left atrium into the left ventricle. No anticoagulants or antiplatelet drugs were administered after surgery. The implants were retrieved 30 days following implantation.

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Conclusions: The excellent results of these initial implantations demonstrate the feasibility of the implantation and cannulation technique, the proper design of the cannula tip and the capability to provide high flow support.

03 IN VIVO PERFORMANCE OF THE INNOVAMEDICA PNEUMATIC VENTRICULAR ASSIST DEVICE
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Objectives: The Innovamedica pneumatic ventricular assist device (VAD) is a new prototype of a simple, low-cost device for hospital circulatory support programs. This study was designed to evaluate the short-term, in vivo performance of this new VAD.

Methods: We implanted the Innovamedica VAD in 6 sheep (weighing 55 to 91 kg). The inflow cannula was placed in the left ventricular apex, and the outflow cannula was anastomosed to the descending thoracic aorta. After heparinization (3mg/kg), we initiated the pump and monitored its hemodynamic performance for 6 hours. We evaluated hematological and biochemical variables at pump initiation and 6 hours later at pump termination. Plasma free hemoglobin levels were assessed hourly.

Results: No complications or device failures were seen in any sheep during the study. The pumps were operated to maintain a blood flow of 4.4 ± 0.8 L/min. During ventricular support, mean arterial blood pressure was 76 ± 15 mmHg. The average concentration of plasma free hemoglobin (corrected for variations in hematocrit relative to baseline) was 8.32 ± 1.5 mg/dL compared with an average baseline value of 8.49 ± 1.5 mg/dL. No biochemical variable changed significantly throughout the study.

Conclusions: The Innovamedica VAD was easy to implant and de-air. During ventricular support, the device maintained a significant proportion of the total blood output. Finally, hemolysis was negligible during the test period, and we saw no thrombus formation or other bioincompatibility problems. Long-term safety and feasibility studies are ongoing at the Texas Heart Institute.

04 ANALYSIS OF FLOW PATTERNS IN THE TOTAL ARTIFICIAL HEART REINHEART®
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Objectives: The Aachen Total Artificial Heart ReinHeart is a double chamber diaphragm pump, which functionally and anatomically replaces the explanted ventricles. Both durability and bioincompatibility for long-term device application remain as major challenges. This study was undertaken to optimize the pump's flow conditions using Particle Image Velocimetry (PIV).

Methods: The ability of newly designed pump chambers to produce a physiological range of flow and pressure was investigated in a mock loop and compared to in vivo results from an acute animal trial. The flow within the pump chambers was quantified using High Speed PIV techniques. The inflow/outflow alignment of single leaflet and bi-leaflet valves was tested to optimize the flow patterns within the chambers. Furthermore we applied new pumping programs. This study was designed to evaluate the short-term, in vivo performance of this new VAD.

Results: The PIV measurements revealed that a bi-leaflet inflow valve should be mounted with the leaflets opening perpendicular to the horizontal plane of the chamber. This configuration produced smooth flow paths, a good washout and low shear stress. However, in an adequate orientation, a single-leaflet valve produced better chamber washout, but higher shear stress. The type/orientation of the outlet valve had limited bearing on the pattern of flow in stroke volume. Finally, a negative pressure in the drive compartment was found to have a high influence on both valve closing behaviour and pump flow. Applying -20mmHg during diastole promoted filling, and thus increased the flow rate by 15% and reduced the acceleration of the blood.
ARTERIOVENOUS FISTULA

05
A COMPARISON OF DIFFERENT ONLINE TECHNIQUES TO MEASURE VASCULAR ACCESS FLOW IN HEMODIALYSIS PATIENTS
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Objectives: In order to accurately follow the vascular access flow (AF) in hemodialysis (HD) patients with a native arteriovenous (AV) fistula, different techniques are applied: the direct blood dilution method (Transonic Systems, the Netherlands), and indirect blood temperature monitoring (BTM) and online clearance monitoring (OCM). The present study aimed to study the impact of blood flow, as well as to compare the access flow results as obtained by the three monitoring techniques.

Materials and Methods: Twenty stable chronic HD patients with an AV fistula, were dialyzed using the 5008H dialysis machine with high flux FX80 (n=14) and FX800 dialyzers (n=6) (Fresenius MC, Germany). All patients were dialyzed in standard HD mode with a blood and dialysate flow of 300 and 500mL/min, respectively, and ultrafiltration rate according to patient’s needs. Transonic, BTM and OCM measurements were performed simultaneously. In ten patients, measurements were repeated for a blood flow Qb of 200mL/min.

Results: Access flow was 1264±664mL/min as determined indirectly with BTM, and was not significantly different from the AF as measured with the Transonic: 1104±607mL/min. The AF’s as calculated using OCM were unreliable and unrealistic. Correlations were found between the AF results with BTM and Transonic (R=0.88, P<0.001). However, BTM tends to show higher AF’s compared to those as obtained with the Transonic, most pronounced for AF higher than 1500mL/min. Comparing the results for Qb200 and Qb300, a correlation (R=0.98 and R=0.94, respectively) and no significant differences were found as performed with the Transonic and BTM, respectively.

Conclusions: The present results indicate that both measuring techniques, BTM and Transonic, give similar results for AV fistula flow in HD patients, with values for BTM slightly but not significantly higher. Furthermore, in case a patient’s dialysis Qb can not be set to 300mL/min, reliable measurement can also be performed in the lower Qb range of 200-300mL/min.

06
ARTERIOVENOUS FISTULA: A REALISTIC NUMERICAL BLOOD FLOW SIMULATION WITH A COMPLIANT WALL
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Objectives: Arteriovenous fistulas (AVF) are subject to numerous pathologies that may be caused by hemodynamic complications such as stenosis initiated by intimal hyperplasia development, and thrombosis. The blood flow is nevertheless very difficult to investigate in these highly disturbed regions using classical imaging or Doppler technique. In a previous work we have presented the numerical simulation of a realistic geometry with a rigid wall. In this study, we propose to numerically model the blood flow in a side-to-end functional AVF with a compliant wall.

Materials and Methods: The AVF geometry originated from our previous work. By using computational fluid dynamics (ANSYS CFX Ltd. USA), the flow patterns and the wall shear stresses were calculated. A time-dependent patient specific physiological mass flow was specified as the inlet boundary condition. As known the AVF is composed of three zones, artery, vein and the anastomoses. Due to the absence of their mechanical wall properties in the literature, we have varied the mechanical compliance properties of the three zones.

Results: Varying the wall compliance property from viscoelastic to rigid has altered the flow results. The artery and the anastomosis showed an important variation in the blood flow and wall shear stress.

Conclusions: The characterization of AVF wall mechanical behavior and stresses are becoming more a need for a realistic experimental and numerical investigation. Credible AVF simulation could be a prognostic tool that can identify blood recirculation zones and locate regions with high and low wall shear stress.
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BIOENGINEERING AND IMAGING

O9 NEED OF IMAGING METHODS FOR BIOENGINEERING OF THREE DIMENSIONAL CELL CULTURE, INCLUDING MATRIX DEVELOPMENT INTRODUCTION

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Regenerative medicine and tissue engineering are fast growing fields especially on implants for human therapy. Nevertheless, the whole process of reactor design and tissue/cell cultivation will be unsuccessful without using proper measuring methods to obtain reliable feedback during the cultivation process. Another point, with regard to clinical applications, is the availability of quality control for the tissue engineered implants. Up to now, engineered constructs are produced of various qualities under non-reproducible and uncontrollable culture conditions, which of a make clearance on the success of the healing process. These are reasons for an intense need of a functional, molecular and structural, imaging of engineered tissue or three-dimensional cell culture in vitro and in vivo. It has been shown that non-invasive imaging methods, such as laser scanning microscopy, offer several advantages compared to conventional fluorescent or staining procedures. These non-invasive techniques appear to be efficient tools for 3D resolved fluorescence imaging, even for the detection of autofluorescent signals in thick and strongly scattering samples. Moreover, the coupling of cell culture devices with appropriate imaging techniques is a very promising approach to fulfill the requirements of online analytical systems in regenerative medicine particularly concerning the PAT initiative of the FDA.

O10 OSTEOCLASTIC BIORESORPTION OF BIOMATERIALS: 3D-IMAGING AND QUANTIFICATION

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Objectives: There is no artificial bone available today. Therefore patients with large defects in their bones due to cancer, trauma, infection or other bone destroying diseases are commonly treated with bone substitute biomaterials. In contrast to living bone, these are dead substances and therefore subject to wear and fatigue. To overcome this potential problem, bioresorbable materials have been developed in the hope that the body will replace them with newly formed bone. The first step of this remodeling process is the bioresorption of the material by osteoclasts. We have developed in our lab different techniques to study the amount of bioresorption of these materials in vitro.

Materials and Methods: Osteoclast precursor cells were isolated from peripheral human blood, purified and cultivated in the presence of the cytokines M-CSF and RANK-L for 4 weeks directly on bone substitute biomaterials to generate human osteoclasts. Osteoclast development was monitored by fluorescence microscopy, offer several advantages compared to conventional fluorescent or staining procedures. These non-invasive techniques appear to be efficient tools for 3D resolved fluorescence imaging, even for the detection of autofluorescent signals in thick and strongly scattering samples. Moreover, the coupling of cell culture devices with appropriate imaging techniques is a very promising approach to fulfill the requirements of online analytical systems in regenerative medicine particularly concerning the PAT initiative of the FDA.

Results: The osteoclast cultures on the biomaterials showed homogeneous layers of multinuclear cells, presenting the typical osteoclast-specific markers like tartrate-resistant acid phosphatase. Light microscopy and raster electron microscopy and threedimensional focus variation microscopy.

Conclusions: The osteoclast cultures on the biomaterials showed homogeneous layers of multinuclear cells, presenting the typical osteoclast-specific markers like tartrate-resistant acid phosphatase. Light microscopy and raster electron microscopy and threedimensional focus variation microscopy. The results of the osteoclast cultures on the biomaterials showed homogeneous layers of multinuclear cells, presenting the typical osteoclast-specific markers like tartrate-resistant acid phosphatase. Light microscopy and raster electron microscopy and threedimensional focus variation microscopy. Moreover, the threedimensional data generated by focus variation microscopy now allow an easy-to-perceive visualization of the resorbed area. This vivid impression might facilitate the understanding of the resorption process.

O11 EVALUATION OF CHONDROGENIC DIFFERENTIATION BY IMAGE ANALYSIS

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Objectives: The organisation of the collagenous network formed by expanded human articular chondrocytes (hAC) or human meniscal stem cells (hMSC) is a critical parameter for the formation of hyaline-like cartilage in vitro. Collagen type II is a major component of cartilage matrix, whereas collagen type VI is specific for the matrix of the chondrons. Collagen type I, formed by expanded hAC and hMSC, lacks in mature cartilage tissue. In this study, expanded hAC and hMSC were differentiated in alginate gel and assessed for the distribution of collagens type I, II and VI in their cell associated matrix (CAM).

Materials and Methods: The expansion of hAC was carried out on bioactive glass (CytoTec 3) and in collagen culture as a control. HMSC were propagated in conventional monolayer culture. Expanded cells were cultivated in alginate gel for 2-3 weeks at reduced oxygen tension (5%) in order to allow the formation of cell associated matrix. For the stimulation of matrix synthesis, recombinant human TGFß and IGF-I were added to the medium. Recovered cells were analyzed for the production of collagen type I, II and VI, respectively, using indirect immunofluorescence, the percentage of collagen producing cells and the distribution of the three collagen types in the CAM matrix were determined.

Results: BMP-7 and IGF-I effectively stimulated the expression of collagen type II by hAC expanded on microcarriers. On the other hand, TGFß and IGF-I combination induced collagen type II synthesis by hMSC. In both cases, collagen type I was also present in the CAM, but its distribution was strongly dependent on the conditions applied during expansion of hAC. Collagen type VI was detected in close proximity to the cells, resembling the organisation of chondrons in native articular cartilage.

Conclusions: Studying the distribution of collagens in the CAM by image analysis is a valuable and possibly predictive tool for the success of cartilage formation in vitro.

O12 TWO-PHOTON TECHNIQUES IN TISSUE ENGINEERING

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Objectives: NIR radiation in the range about 800 nm is less absorbed by biological tissues. Femtosecond pulsed Ti:Sa lasers provide ultrashort NIR laser pulses with high energy to trigger two-photon effects. Especially in life sciences research two-photon techniques obtain an ever-increasing importance. We introduce two laser applications for tissue engineering: the autofluorescent visualization of cells within 3D-scaffolds after two-photon excitation and the manufacturing of 3D-structured hydrogel-like scaffolds by triggering a radical polymerisation processes within polymerisable solutions.

Materials and Methods: Primary bovine chondrocytes were cultivated on different collagen I/II scaffolds using a flow chamber system with an online coupled two-photon laser scanning microscope (2PLSM). During the incubation the cell populations were hydrostatically stimulated. The selective visualization of unlabelled cells and scaffolds was achieved by the spectral autofluorescence imaging and fluorescence lifetime imaging (FLIM). To modify scaffold mediated effects on cell growth and cell differentiation hydrogel-like scaffolds with well defined 3D structures were generated by two-photon polymerisation (2PP) using methacrylated hydrogel monomers.

Results: It could be shown that the spectral autofluorescence imaging provides spatially resolved data for the noninvasive online control of the tissue growth. It has been shown that non-invasive imaging methods, such as laser scanning microscopy, offer several advantages compared to conventional fluorescent or staining procedures. These non-invasive techniques appear to be efficient tools for 3D resolved fluorescence imaging, even for the detection of autofluorescent signals in thick and strongly scattering samples. Moreover, the coupling of cell culture devices with appropriate imaging techniques is a very promising approach to fulfill the requirements of online analytical systems in regenerative medicine particularly concerning the PAT initiative of the FDA.

Conclusions: Two-photon techniques provide powerful tools for both the noninvasive online visualization of 3D-cell-scaffold constructs and the structuring of 3D environments based on native ECM components. The techniques are not limited to biomedical applications but also suitable for microsystem technological applications (e.g., BioMEM).
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O12 RAMAN SPECTROSCOPY AS A NON-INVASIVE TOOL FOR QUALITY AND STERILITY ANALYSIS OF TISSUE ENGINEERING PRODUCTS
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Objectives: The non-invasive analysis of cells is a major challenge in the field of cell biology and tissue engineering. Raman spectroscopy with its key advantage to analyze cells under in vivo conditions could overcome this limitation. A bottleneck in the production process of tissue engineering products is the sterility control with its long cultivation times. The rapid, reagent free and non-invasive characterization of cells and microorganisms by Raman spectroscopy increases the efficiency of quality and sterility control of tissue engineering products. Our work focuses on the qualification and establishment of this technique.

Materials and Methods: Cells were purchased by ATCC as well as isolated from porcine and human material respectively. Microorganisms were purchased by DSMZ. Sample preparation for Raman measurements of cells and microorganisms consist in suspension in medium. The new developed micro-Raman spectrometer is coupled with a fluorescence microscope. A 785nm diode laser is focussed on the sample. Detection is done with a charge-coupled device and a spectrophotograph. The processing and analysis of data is carried out with software like Opus® and the Unscrambler®.

Results: The discrimination of several cell types by Raman spectroscopy as well as the determination of variances during dedifferentiation of cells is possible and confirmed by immunohistological staining. Diverse microbial organizations of cells within porous matrices by combining Fourier domain optical coherence tomography (FDOCT) and broadband impedance spectroscopy is performed with a dielectric probe and impedance analyzer to gather additional cellular information.

Conclusions: In order to establish an economic production process as well as a quality and sterility control of tissue engineering products, respectively, the rapid and non-invasive characterization of living cells and microorganisms is necessary. In a first step it was shown that the characterization of cells and microorganisms in suspension is possible. Further work needs to be done on the analysis of cells embedded in a scaffold.

O14 COMBINED BROADBAND IMPEDANCE SPECTROSCOPY AND FOURIER DOMAIN OPTICAL COHERENCE TOMOGRAPHY TO MONITOR THREE-DIMENSIONAL CELL STRUCTURE
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Objectives: To assess non-invasively and in real time the three-dimensional organization of cells within porous matrices by combining Fourier domain optical coherence tomography (FD OCT) and broadband impedance spectroscopy (BIS).

Materials and Methods: Broadband interferences resulting from the recombination of in-depth scattering events within the sample and light from a reference arm are measured as a modulation of the spectrum generated by a superluminescent laser diode (800nm FWHM 90nm). Fourier transform allows in-depth localization of the scatterers, and the 3D microstructure of the sample is reconstructed by raster scanning. Simultaneously impedance spectroscopy is performed with a dielectric probe and impedance analyzer to gather additional cellular information.

Results: A combined BIS-FDOCT system allowing an axial resolution of 5micrometer in the tissue and measurements over the range 1MHz-1GHz has been developed. Alginate matrices have been characterized in term of porosity and interconnectivity. Matrices seeded with stem cells have been monitored without the use of labelling agent.

Conclusions: We have developed an optical system that will be instrumental to optimize the biochemical parameters leading to tissue development within three-dimensional porous matrices.
OBJECTIVES: This work aims at developing a hybrid circulatory model (a numerical model merged with a physical one - hydraulic or electrical) that can be used to investigate the hemodynamics of the IABP. We evaluate IABP effects as a function of its timing and selected circulatory and ventricular variables.

Materials and Methods: The lumped parameter computational model consists of left and right hearts, systemic, pulmonary and coronary circulation. The hybrid application is based on physical-electrical models. The computational model was transformed into hybrid by replacing systemic arterial circulation with an electrical model to connect an IABP. The IABP was reproduced electrically as a zero average flow generator. Endocardial viability ratio (EVR), Cardiac Output (CO), systolic and diastolic pressures were analysed vs IABP filling and emptying times, ventricular EEmax (1-5 mmHg cm-3) peripheral resistance and arterial compliance. All experiments were conducted comparing the selected variables before and after IABP start.

Results: Changes in IABP and circulatory parameters influence all the considered variables. In general, IABP assistance produces lower percentage changes in the selected variables increasing peripheral resistance from 1300 to 2000 g·cm-4·sec-1. However, IABP effects on EVR (0.9 to 1.2) are higher in experiments. In the next phase a physical-hydraulic hybrid model will be used. The developed hybrid model provides a platform to perform experiments in stable and repeatable circulatory conditions. The advantage of this set up lies in the possibility of studying a real physical device and measuring its effects on the whole circulatory system, represented by the computational model. The results are realistic, physiological and further provide evidence the hybrid model can be used as an alternative to animal experiments. In the next phase a physical-hydraulic hybrid model will be used.

ONE DAY ON THE LIVER (II)

O18

STUDY OF MASS TRANSFER THROUGH ALGINATE BEADS FOR FLUIDIZED BED BIOARTIFICIAL LIVER

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Objectives: Liver is a complex organ ensuring numerous essential metabolic functions. Up to now, liver transplantation only can efficiently treat these end-stage pathologies. The expanding gap between the number of patients on waiting list and the number of liver transplants confirms the challenge for an alternative solution to treat such patients. The Fluidized Bed BioArtificial Liver (FBBAL) hosting hepatic cells in alginate beads should be a promising solution if correctly dimensioned to optimize the mass transfer and thus cell viability and functions. This study presents experimental and theoretical works to explain the metabolic cell activities of cells entrapped into different alginate beads (600 and 1000 µm) and placed in the FBBAL system.

Materials and Methods: The conservation equations described mass transfer in the FBBAL system. The mass transfer coefficients and the “biological reaction” rates could be determined by implementing the supernatant concentration measured during experiment in our mathematical modelling using the dimension-less Sherwood number.

Results: The mass transfer coefficients and “biological reaction” rate for three different solutes of interest synthesized (albumin and alpha-foetoprotein) or consumed (glucose) by C3A cells were successfully determined. The reduction of bead diameter from 1000 µm to 600 µm did not induce a modification on the internal diffusion following the dimension-less analysis.

Conclusions: Finally, this mathematical tool can be a great help to scale up, and more specifically to calculate the amount of cells, the volume of alginate beads and culture medium needed in extracorporeal systems to replace liver functions.
MATURATION OF HEPG2 CELLS IS CLOSELY INFLUENCED BY THE MICROCHANNEL HEIGHT

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Objectives: Microfluidic systems provide valuable insights into tissue morphogenesis and in vitro stabilization of hepatocyte differentiation because of the large surface-to-volume ratio and behaviour similar to in vivo. Despite the efforts over two decades to define in vitro systems that can replace animal testing, an improved understanding of the interactions between cells and their constrained microenvironment will result in improved cell culture techniques and provide useful insights into how cells reorganize and communicate. Therefore, we focused on the exploration of the relationships between an array of cell activities and microchannel geometry.

Materials and Methods: Kinetics of HepG2 cells activities in conventional cell culture polystyrene dishes to those in PDMS microfluidic cell culture chambers of different heights were compared by using a non-invasive in vitro cell-based multi-components analysis method. Briefly, albumin secretion and glucose consumption were daily measured from culture media, while cell viability, morphology and cell membrane integrity were analyzed by using fluorescent dyes or indicators.

Results: We identified important and fundamental differences between PDMS micro-scale culture systems and polystyrene dishes. The results of glucose consumption and albumin secretion assays indicated that HepG2 cells were able to more effectively modulate their environment in micro-scale cultures than in macro-culture systems, while growth rates were lower within microchannels. In Microsystems, the peak of albumin secretion rate was reached sooner when the microchannel height was reduced, suggesting that cell activities are closely influenced by the height.

Conclusions: Reducing PDMS microchannels height provide promising conditions for long-term and stable cell growth, leading to improved structural organization and functionalities. The close influence of the channel height might be explained by an accumulation of functional soluble factors in the diffusion dominant microchannel environment.

INFLUENCE OF HEMODYNAMICS ON THE FORMATION OF AN INTRALUMINAL THROMBUS IN ABDOMINAL AORTIC ANEURYSMS

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Objectives: Thrombosis is typically observed in abdominal aortic aneurysms (AAA). The potential influence of hemodynamic forces on thrombosis has long been recognized, but it has mostly been studied in vessel geometries that induce abnormally high levels of shear stresses (e.g. stenoses). The purpose of the study is to investigate how hemodynamic factors could lead to the formation of an endoluminal thrombus in AAAs. More precisely, we will characterize the magnitude of the fluid stresses acting on circulating platelets and the time of exposure in the dilatation.

Materials and Methods: The trajectories of fluid particles are calculated using a Lagrangian particle tracking method applied to previously obtained velocity fields. Results of particle tracking conducted on in vitro measurements of velocity fields in AAAs are compared with others obtained from a numerical simulation.

Results: We show that the flow structures within the aneurysm tend to convect platelets towards the wall, which increases their probability of deposition onto the wall. The number of cells convected towards the wall increases with the aneurysm dilatation ratio. These platelets are entrained into regions of slowly recirculating flow, where they experience long residence times and low hemodynamic stresses.

Conclusions: The long residence times, low flow conditions and convective patterns towards the wall are hypothesized to be the main factors contributing to the thrombus formation in AAAs. Thrombosis within AAAs is therefore thought to be linked to platelet aggregation through fibrinogen polymerization.

VISUALIZATION OF THROMBUS FORMATION ON PIPE ORIFICE FLOWS

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Objectives: To suppress the hemolysis and avoid the thrombus is very important and serious problem in developing artificial organs, especially rotary blood pumps and stent. In this investigation, the thrombus formation on a pipe orifice flow with blood plasma is visualized by high speed camera, and done by PIV construct the prediction model of thrombus on shear flow by normal FDM (Finite Difference Method) or other method (lattice Boltzmann method).

Materials and Methods: Using the five transparent orifice configurations made of acryl resin, the flow is visualized by laser sheet light in the blood plasma circuit. In this case, the flow rate is 5 L/min and flow type is pulsatile, the total pressure loss in the circuit is 300 mmHg for every configuration by using additional smoothing resistance. In this experiment, we focus on the process of thrombus formation on the surface of acryl resin. Then we have both coating and no-coating on the acryl resin, so that there is difference of adhesion force on the wall between these conditions. Once accelerating the thrombus formation of blood plasma by using the aggregation drug, the protein and protein in the flow can be visualized by LED light sources. By image processing of the raw movie, the sequential image of the thrombus formation in the flow can be obtained.

Results: The thrombus formation is found to begin at the center of the orifice. The history of averaged brightness level is affected by changing orifice configuration. This means that effects of the shear stress distribution such as maximum shear stress on the thrombus formation, especially thrombus rate at the wall, are large. The effects of wall adhesion force on the wall is also confirmed by changing the thickness of coating layer on the acryl resin.

Conclusions: The thrombus formation in the blood plasma flow was visualized, and the effects of shear stress distribution and adhesion force of the wall on the thrombus formation were estimated for constructing CFD model of thrombus prediction.

INVESTIGATIONS OF A COUNTERPULSATION HEART ASSIST DEVICE BY PIV AND WALL-PIV

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Objectives: The investigation of blood pumps for regions of blood stagnation allows a first estimation of the risk of thrombus formation. The pump under consideration is a novel long-term implantable counterpulsation device (CPD). The CPD is a single port, valveless 32mL stroke volume blood chamber. Attached to the subclavian artery, it can be implanted subcutaneously on the right anterior chest, similar to a pacemaker.

Materials and Methods: The investigation of the flow inside the CPD was done by Particle Image Velocimetry (PIV) to obtain the flow field in the central plane and Wall-PIV to obtain the near wall flow. Both methods were realized on a simplified mock circulation, consisting of a Windkessel and the blood pump. Filling time was 600ms plus a hold time of 850ms. The emptying time was set to 250ms. This corresponds to a 1:1 operation mode, which is more prone for thrombus formation. The pressure head was set to 90mmHg. As a test fluid we used a mixture of water and glycerin, simulating a blood viscosity of 3.5mm²/s. Images were recorded with a MotionPro X3 (Redlake Inc., USA) camera with 2000fps.

The central plane measurement setup allows an insight into the general flow pattern of the pump. For illumination we used a Quantum CW-laser at 0.65W. To investigate the flow along the curved walls of the CPD, we used the Wall-PIV technique developed in our laboratory. Two LED-reflection sources and a molecular dye at 0.3g/L (Patent blue V) allow the near wall region illumination with restricted light depth penetration.

Results: During ejection phase the fluid flows towards the port of the CPD without recirculation. A steady rotating vortex, extending over the complete blood pump, with a permanently moving center, is observed during filling phase and hold time.

Conclusions: The investigated 1:1 mode has a good washout of the whole pump volume. Regions of stagnation are inhibited by a persistent steady rotating vortex. Due to this pump flow we expect a low risk of thrombus formation.

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O25

MODEL-BASED NUMERICAL ANALYSIS OF PLATELET ADHESION, THROMBUS GROWTH AND AGGREGATION FOR ASSIST DEVICES

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Objectives: Thrombosis and thromboembolism are among the leading causes for mortality in patients who depend on artificial organs. In order to be able to predict platelet behaviour it is necessary to consider both the flow conditions inside the device and the thrombogenic properties of the blood-contacting surfaces. Mathematical modelling of thrombotic reactions is established and validated in test cases. Aim of this study is to experimentally evaluate and computationally simulate platelet activities under the influence of well-defined shear rates for cylindrical gap flow, laminar flow in a rectangular channel and a stagnation point flow. The application of this model is directed towards the design of left ventricular assist devices, hemodialysis and gas exchange devices.

Materials and Methods: A mathematical model of platelet activation, adhesion and aggregation has been implemented into a finite element CFD code. The approach is based on the advective and diffusive transport equations for resting platelets, activated platelets and platelet released agents, and on the diffusion and collision efficiency terms describe the interactions between them. Experiments with citrated whole blood are performed in a rectangular flow chamber as well as in a Taylor-Couette system for laminar and for Taylor vortex flow. The activation and drop of single platelets, adhesion and aggregation are measured.

Results: The thrombosis model was applied to different three-dimensional test cases of clinical significance. The numerical simulation results based on physiologically relevant values for the model parameters were successfully validated against experimental data. Regions and flow conditions with a high potential for thrombus growth could be identified.

Conclusions: The numerical method shows good agreement with measured platelet reactions and adhesion for different test devices. The model can be used for the analysis and prediction of thrombus growth in artificial organs.

O26

IMPLANTATION OF CARDIOVASCULAR DEVICES: SIGNIFICANCE AND FUTURE ROLE OF IMAGE GUIDANCE

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Purpose: To present the future potential of innovative imaging modalities and their impact on optimization of current, and accordingly, establishment of new implantation techniques for cardiovascular devices.

Materials and Methods: In a cardiovascular catheter lab, the “golden standard” of imaging is fluoroscopy, combined with digital subtraction angiography (DSA). In special cardiovascular applications, contrast enhanced computed tomography (CT) or magnetic resonance angiography (MRA) may be advantageous for the guidance of instruments and implant. Ultrasound 3D reconstruction is an important feature, offering a quasi-real-time stereical orientation of the relation between anatomy and implant. Recent developments combine DSA with CT, by rotating the C-arm laterally around the patient while collecting an array of equally spaced 2D x-ray projection images, and then using algorithms to reconstruct a three-dimensional image. A brand new development of a robot suspended rotating C-arm offers a tremendous increase of versatility for the OR setting. Further technical achievements comprise hybrid imaging (e.g. x-ray, echocardiography, and MRA for percutaneous heart valve placement) and the integration of navigation systems (optical or electromagnetic) into the workflow of image guidance.

Results: The thrombosis model was applied to different three-dimensional test cases of clinical significance. The numerical simulation results based on physiologically relevant values for the model parameters were successfully validated against experimental data. Regions and flow conditions with a high potential for thrombus growth could be identified.

Conclusions: The numerical method shows good agreement with measured platelet reactions and adhesion for different test devices. The model can be used for the analysis and prediction of thrombus growth in artificial organs.

O27

FIRST EXPERIENCE WITH AN FDA CRITICAL PATH INITIATIVE: CFD AND HEMOLYSIS


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Objectives: In 2008, the FDA started a Critical Path Initiative for Computational Fluid Dynamics (CFD). The primary goal of this project is to accelerate the CFD use in the safety assessment of medical devices. Particular attention is being paid to blood damage. A Guidance Document for Computational Fluid Dynamics (CFD) was published and validated and CFD methods evaluated in round-robintesting should be created. The round robin 1 test is an axial symmetric nozzle incorporating tubes of two diameters (4 mm and 12 mm) with a cone connecting the two diameters on one end, and a sudden change in diameter in the other. Both flow directions (sudden expansion and conical diffuser) and five flow rates should be investigated (Re=500, 2000, 3500, 5000, 6500). A report of our experience with the round robin 1 test is presented here.

Materials and Methods: Flow simulations in the FDA proposed nozzle were performed using the CFD program FLUENT®. An unstructured mesh using 1,100,000 hexahedrons was generated. The mesh independence study included meshes between 300,000 and 1,800,000 cells. Simulations with Reynolds numbers over 3000 included the RANS k-ω-turbulence model. Hemolysis was modelled using the mass transport equation based solution with a source term as a function of shear rate, which is based on the corrected power law Giersiepen equation and includes flow history dependence. This method was developed in our group (Goubergrits L, Expert Rev Med Devices 2008; 3:6:527-31). Damage of erythrocytes was quantified as a relative index of hemolysis (RH) normalized with a hemolysis index calculated for conical diffuser flow direction with Re=3500 and evaluated at the outlet.

Results: The mesh independence study revealed significant difficulties in achieving convergence of the recirculation region length caused by a sudden expansion. Differences between both flow directions were about 6%. The resulting RH for the five Re numbers are 0.07, 0.64, 1.00, 0.86 and 0.97.

Conclusions: Based on our study we conclude that the design of the round robin 1 test needs some improvements regarding nozzle geometry and choice of flow rates.

ONE DAY ON THE LIVER (III)

O28

ARTIFICIAL LIVER SUPPORT SYSTEM REDUCES INTRACRANIAL PRESSURE MORE EFFECTIVELY THAN BIOARTIFICIAL SYSTEM – AN EXPERIMENTAL STUDY

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Materials and Methods: A surgical devascularisation model of ALF was used in pigs (35-40 kg). The elimination therapy started after hypoglycemia (<3.5mmol/L) onset and the biochemical parameters (bilirubin, ammonia, lactate, glycemia etc.) as well as the intracranial pressure (ICP) were monitored during the 12-hour experiment. Of the total 34 pigs with ALF, 16 animals were treated by fractionated plasma separation and absorption (FPSA, Prometheus, Fresenius), 10 by bioartificial liver (BAL, O.liver Performer, Rand) and 8 created the control group. For the statistical analysis Bartlett’s test was first used to compare the variance between the two systems in pig experiment.

Results: New technical achievements in imaging techniques and optimization of their integration into the clinical workflow offer the potential for innovative, minimal-invasive implantation procedures for cardiovascular devices.
Abstracts: XXXVI Annual ESAO Congress, 2-5 September 2009, Compiègne - France

ICP values were reduced significantly in pigs treated by FPSA compared to BAL: the 10h 21.9 vs. 25.3, the 11h 22.5 vs. 26.7, the 12h 24.0 vs. 33.0 (p<0.005) mm Hg.

Conclusions: The artificial liver support system FPSA only significantly reduces the ICP values. It seems that the FPSA has a positive impact on other parameters of ALF.

O29 IS THE EFFICACY OF MARS TREATMENT IN ACUTE-ON-CHRONIC LIVER FAILURE RELATED TO PRIMARY LIVER DISEASES?
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Objective: To evaluate the efficacy of MARS (Molecular Adsorbent Recirculating System) in the treatment of acute-on-chronic liver failure caused by different primary liver diseases.

Patients and Methods: We have enrolled and treated 80 patients affected by liver failure secondary to: chronic hepatitis C (28) group (g) A, chronic hepatitis B (8) g. B, alcoholic liver disease (21) g. C, primary biliary cirrhosis (7) g. D, hepatorenal syndrome (8) g. E, severe cholestasis after liver transplantation (I.t.) (2) g. F, recurrent chronic hepatitis C after I.t. (2) g. G, autoimmune chronic hepatitis (g. H), animal xenograft (g. I). Patients were divided in 8 groups: I, 35 patients included in the study study; the pruritus refractory to pharmacological therapy and showed scratching skin lesions. Treatment modalities: 2-7 daily sessions according to the patient needs; session time 5 hours; blood flow 220±20 mL/min; albumin flow 150±10 mL/min; bile flow (in the albumin dialysate flow) 500±50 mL/min; heparin 750±1500 u/h; blood analyses: bilirubin, bile acids, ammonia, urea, creatinine, plasma electrolytes, base-acid balance at the beginning and at the end of each session and alkaline phosphatase, cholinesterase, prothrombin activity, AST, ALT, GGT before and after MARS cycle and one month later.

Results: At the end of each treatment total bilirubin, bile acids and ammonia fell of 27±5%, 40±6%, 54±14%, respectively. After the MARS cycle cholestasis parameters and liver function tests improved with a significant decrease of bilirubin, bile acids, ammonia, alkaline phosphatase (p<0.001) and a significant increase of cholinesterase and prothrombin activity (p<0.002), in 65 patients: 24/28 in gr. A, 6/8 gr. B, 19/21 gr. C, 6/7 gr. D, 5/8 gr. E, 2/2 gr. F, 1/2 gr. G, 1/2 gr. H, 1/2 gr. I. Severe pruritus disappeared in all cases after the third treatment.

Conclusions: The temporary success of MARS treatment is related to the potential recovery of the liver function and to the clearance of the cholestasis more than to primary liver disease.

O30 FPSA DOES NOT IMPROVE HEMODYNAMIC PARAMETERS IN ACUTE LIVER FAILURE IN PIGS
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Objectives: Acute liver failure (ALF) is a quite rare, but severe disease with very unfavorable prognosis. The onset of ALF is accompanied by significant hemodynamic instability. The aim of our study was to evaluate the influence of the treatment by Fractionated Plasma Separation and Absorption (FPSA) on hemodynamics in ALF in a controlled experimental trial.

Materials and Methods: ALF was induced in 21 pigs by surgical devascularisation. The animals were divided into two groups. In 14 pigs the treatment by FPSA was started after the onset of ALF, documented by hypoglycemia (<3.3 mmol/L), and the treatment lasted 6 hours. The control group was in the 6, 9 and 12 hour comparing to the initial values. We did not find any significant difference comparing the MAP (Mean Arterial Pressure) and SVRI values (p>0.05) in the FPSA-treated group and the control group.

Results: We found the significant decrease (p<0.05) of SVRI (Systemic Vascular Resistance Indexed) in both groups in the 3, 6, 9 and 12 hour of the experiment and the significant increase of HR (Heart Rate) and Cardiac Index (CI).

Conclusions: There is no significant improvement of important hemodynamic parameters in the treatment of ALF using the new extracorporeal eliminating method FPSA. The FPSA device provides a significant attenuation of the rising ICP in ALF.

O31 USE OF A LIVER BIOCHIP AS A TOOL IN TOXICOLOGICAL STUDY
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Objectives: In the present paper we describe and compare the cell cycle distribution and the metabolism activities of hepatocarcinoma liver cells in microchips and Petri dishes. Current developments in tissue engineering and microtechnoology fields have allowed the proposal of newpertinent tools, also called cell microchips, to investigate in vitro toxicity. In the framework of the continued effort to improve both the "in vitro" cerebral perfusion, the purpose of these cell microchips is to mimic organs in vitro in order to refine in vitro culture models and to ultimately reduce the animal testing. To validate this approach, it is necessary to assess the proliferation and the cellular product was placed in those cell microchips when compared to conventional cell culture techniques.

Materials and Methods: The microchip consists of polydimethylsiloxane (PDMS) microchannels interconnected with a fluidic network that allows 150 µL continuous feeding and waste removal. The microchips is serially connected to a peristaltic pump and a culture medium tank. For comparative purposes, Petri dishes coated with PDMS were also prepared. The hepatocarcinoma HepG2/C3a liver cells were inoculated at 300 000 cells/microchip. The perfusion flow rate was 10µL/min. The data are reproduced six times.

Results: The growth of the cells and the metabolism have been followed every 24 hours and during 96 hours. The cellular stress was monitored by the cell cycle distribution using a flow cytometer whereas the glucose and the albumin concentrations were monitored by commercially available detection kits.

Conclusions: The comparison between both models demonstrates that microchip will be suitable for in vitro cell culture with a good cellular proliferation, a good activity of synthesis and no abnormal cellular cycle.

MODELLING CARDIOVASCULAR DEVICES
O32 THE IMPACT OF OUTFLOW CANNULA POSITIONING ON CEREBRAL PERFUSION DURING CARDIAC SUPPORT: A CFD STUDY
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Objectives: Device cannulation to the Cardiovascular (CV) System is an important consideration for Cardiac Support. There are different approaches for cannula positioning inside the aorta: some devices (HEART MATE II) return blood via the ascending aorta, while others (JARVIK 2000) place the outflow cannula in the descending aorta. A CFD study was initially undertaken to study the impact of both approaches on cerebral perfusion.

Materials and Methods: A 3-dimensional model of the human CV-System was reconstructed from realtime CT/MRI data, including aorta, carotid, subclavian and vertebral arteries. In a CAD model, a conventional outflow vessel was placed in different positions inside the ascending and descending aorta. A transient numerical simulation was performed for each cannula position, assuming a pulsatile cardiac output of 3 L/min from the heart and 2 L/min continuous support from a standardised assist device. Cerebral perfusion and wall shear were analysed for all positions.

Results: Wall shear is not affected by the outflow cannula position. For cannulation to the ascending aorta, a negative flow in the outgoing arteries can be observed during diastole, so blood is withdrawn out of these supplying vessels. Therefore, the average cerebral perfusion is about 40% lower for that approach. Cannulation to the descending aorta provides good flow to all vessels for the whole cycle.
Conclusions: This work provides a method to compare different cannulation approaches for Cardiac Assist Devices and hence find the best cannula position for each single device, dependant on support conditions such as cardiac output. First simulations suggest that cannulation to the descending Aorta provides a better flow to the distal vessels. Nevertheless, this has to be investigated and validated in further studies, regarding the exact boundary conditions and outflow cannula positions for different Assist Devices.

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**O33 HYDRODYNAMIC ANALYSIS OF NEW DISPERSIVE AORTIC PERFUSION CANNULA**

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Objectives: Edwards Research Medical EZ Glide Aortic Perfusion Cannula TM (EZ Cannula) was developed to attenuate flow in the aorta and avoid atheroembolism due to jet flow.

**Materials and Methods:** Hydrodynamic analysis of the EZG Cannula was performed using particle image velocimetry in mock glass aortic perfusion and fluid structure interaction (FSI) model of the MV. Differences between linear and non-linear elastic material properties have been examined, alongside isotropic and anisotropic material definitions. Modelling of the FSI MV model in both the simple and ventricular chambers.

**Results:** The numerical simulation showed good agreement (<6%) with the experimental data for different mass flows and constant gas partial pressure at the inlet of the MicroMox. Good agreement (~6%) could also be achieved for experimental data for different mass flows and constant gas partial pressure in both the simple and ventricular chambers.

**Conclusions:** Trileaflet mechanical heart valve prostheses provide superior hydrodynamics, a more natural valve behavior and a significantly lower thrombogenic potential than currently available mechanical heart valve prostheses.

**O35 THE TRIFLO TRILEAFLET MECHANICAL HEART VALVE: DESIGN AND IN VITRO PERFORMANCE**

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Objectives: Trileaflet mechanical heart valves combine the central flow characteristic of biological with the durability of mechanical prostheses. In this study, the design process and the in vitro characteristics of the new Triflo trileaflet mechanical heart valve prostheses will be presented.

**Materials and Methods:** Design optimization of the Triflo valve was performed by means of enlarged model studies, mainly focussing on an early and soft closure and a good washout of whole blood. A non-linear blood flow model was implemented in transient flow simulations. Opening and closing behaviour were analyzed quantitatively by high speed video recording and subsequent image analyses. Thrombogenicity was evaluated in vitro in a pro勃勃atory blood test system.

**Results:** The optimized Triflo valve design showed a favourable washout, lower pressure drop and regurgitant volumes, more evenly distributed flow profiles and lower shear stress distributions in comparison to commercially available valves. Furthermore, the opening and closing behavior of the valve compared well to that of biological prostheses, which was in clear contrast particularly to the closing of other mechanical valves. The in vitro thrombogenicity was superior, as thrombus formation appeared significantly later and to a minor extent than in other mechanical prostheses.

**Conclusions:** The Triflo trileaflet mechanical heart valve prosthesis provides superior hydrodynamics, a more natural valve behavior and a significantly lower thrombogenic potential than currently available mechanical heart valve prostheses.

**O34 MODELLING AND SIMULATION OF THE MITRAL VALVE UNDER PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS: A PRIMER STUDY**

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Objectives: The goal of this study is to develop a parametric model of the mitral valve (MV) which will assist clinicians in the investigation of MV disease and associated problems such as: MV stenosis, MV insufficiency and MV prolapse, all of which can occur alone or in combination. Following the concept of “predictive medicine”, this model serves as a foundation for the development of further patient-specific models. Here we report a structural and fluid structure interaction (FSI) model of the MV.

**Materials and Methods:** Simulation of valve dynamics has been performed using a parametric finite element model based upon anatomical dimensions and valvular features reported in literature. Application of systolic pressure (120 mmHg) to the model has been simulated using the numerical code LS-DYNA. Differences between linear and non-linear elastic material properties have been examined, alongside isotropic and anisotropic material definitions. Modelling of the FSI valve will also be investigated using beam elements and shell elements. The interaction of blood with the MV will be simulated using FSI, flow through the MV into a simple chamber will be performed with the aim of integrating the model into a ventricular shaped chamber.

**Results:** The current results of the linear elastic structural model compare well to previous models, with the von Misses stresses in the centre of the anterior and posterior leaflets 0.39 and 0.25 MPa, respectively. Singular stresses locations were found to occur at the location of chordal attachment. Differences in the valve closure between the two chordal models and the different material models will be presented, alongside the preliminary results of the FSI MV model in both the simple and ventricular chambers.

**Conclusions:** This model allows the simulation of the MV under normal and pathological conditions such as MV prolapse. It forms the basis of a tool which will allow clinicians to further understand the best course of action under different pathological scenarios.

**O36 AN VALIDATED CFD MODEL TO PREDICT O2 AND CO2 TRANSFER WITHIN HOLLOW FIBER MEMBRANE OXYGENATORS**

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Objectives: Hollow fiber oxygenators provide gas exchange to and from the blood during heart surgery or lung recovery. Minimal fiber surface area and optimal gas exchange rate may be achieved by optimization of hollow fiber shape and orientation. In this study, a CFD model is developed and validated for porcine blood with a self developed micro oxygenator (MicroMox).

**Materials and Methods:** The MicroMox was designed such that bundle of 120 microtubes is exposed to the blood flow and oxygen diffusion is calculated using finite element methods. The entire bundle of 120 fibers. A fine mesh was generated in order to resolve the high gradients during mass transport. A non-newtonian blood model was used in transient flow simulations. Opening and closing behaviour were analyzed quantitatively by high speed video recording and subsequent image analyses. Thrombogenicity was evaluated in vitro in a prop勃勃atory blood test system.

**Results:** The optimized Triflo valve design showed a favourable washout, lower pressure drop and regurgitant volumes, more evenly distributed flow profiles and lower shear stress distributions in comparison to commercially available valves. Furthermore, the opening and closing behavior of the valve compared well to that of biological prostheses, which was in clear contrast particularly to the closing of other mechanical valves. The in vitro thrombogenicity was superior, as thrombus formation appeared significantly later and to a minor extent than in other mechanical prostheses.

**Conclusions:** The Triflo trileaflet mechanical heart valve prosthesis provides superior hydrodynamics, a more natural valve behavior and a significantly lower thrombogenic potential than currently available mechanical heart valve prostheses.
Conclusions: A validated model for the prediction of gas exchange in hollow fiber oxygenators could be established. It can now be used to optimize fiber arrangements and thus the efficiency of hollow fiber oxygenators.

O37

COMPUTATIONAL STUDY OF A NOVEL AXIAL FLOW PUMP AS LVAD

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Objectives: Axial flow pumps have proved to be viable long-term circulatory assist devices. The overall objective of this work is to develop an axial flow pump, to be placed at the aortic root, supporting the flow to the coronary arteries during diastole and towards the periphery during systole. The aim of this study is to design a bidirectional blade that generates flow and pressure rise when rotated in either direction.

Materials and Methods: Stepanoff’s design method for unidirectional axial flow pumps was used to design two unidirectional blades, one for each direction. A merge of the two NASA profiled blades lead to a symmetric bidirectional profile. The blade angle, measured from the rotating axis, varied from ~70° at the hub to ~50° at the hub. 2D and 3D computational fluid dynamics (CFD) models were employed, with constant rotational speed of 1,500 rpm, to optimize the performance and potentially evaluate blood trauma.

Results: Pressure rise and blade loading profiles of 2D and 3D simulations were compared. A systolic head of 2.7 kPa and 7.7 kPa, and a diastolic head of 2.4 kPa and 4.5 kPa were obtained in 2D and 3D, respectively. The theoretical pressure rise is 18 kPa, while the average simulated pressure rise is ~14% (2D) and ~34% (3D) of that calculated theoretically. Discrepancies between theoretical and simulated pressure rises are expected as friction and hydraulic losses are neglected in the theoretical calculation.

Conclusions: A first design for a bidirectional impeller is established and characterized computationally. Analysis of the pressure blade loading profiles, sectioned between hub and tip, confirmed generation of a pressure rise, leading to a good balance in performance between the forward and backward direction. Although the rotational speed was kept constant, it is expected that the diastolic rotational speed can be reduced as, compared to sylorole, more time will be available to deliver a smaller flow volume.

DIALYSIS CATHETERS

O38

SCLEROSING ENCAPSULATING PERITONITIS AS A LIFE-THREATENING COMPLICATION OF CONTINUOUS AMBULATORY PERITONEAL DIALYSIS

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Objectives: Sclerosing encapsulating peritonitis (SEP) is rare and dramatic complication of continuous ambulatory peritoneal dialysis (CAPD). It is characterized by progressive inflammatory process resulting in thickening and constricting of peritoneal membrane, thereby compromising the bowel motility. Treatment options include intestinal enterolysis, immunosuppressive treatment and total parenferal nutrition with varying success.

Patients and Methods: This report reviews our experience in the diagnosis and treatment of patients with SEP. Clinical characteristics, risk factors, treatment modality and outcome were recorded.

Results: Out of 520 CAPD patients who entered our peritoneal dialysis program until December 2008, seven patients developed SEP (1.34%). Male-to-female ratio was 2:5 with mean age of 56 (range 37 to 72) years. The median duration of CAPD was 117.57 (range, 72-149) months. All patients were treated with CAPD for at least 6 years at the time of SEP diagnosis, with four of them on CAPD longer than 11 years. Clinical presentation of SEP includes symptoms suggestive of intestinal obstruction in two patients, bloody ascites after transfer to hemodialysis for ultrafiltration failure in two patients, radiological signs of peritoneal calcifications during the pretransplant examination in two patients and perforation of appendix in one patient. All patients experienced decreased solute or fluid removal before the diagnosis of SEP. Results of PET test demonstrated high transporter characteristics in 5 patients, while both male patients were high average transporters. Patients had 3 to 4 previous episodes of peritonitis which in two of them preceded the diagnosis of SEP. Causative microorganisms include S. epidermidis in 9 cases, S. aureus in 2, P. rettgeri in 1, S. viridans in 1, E. faecalis in 1 and E. coli in 3 cases. In two cases peritonitis was due to multiple microorganisms. One patient had four and one patient experienced two episodes of sterile peritonitis.

After the diagnosis of SEP, patients were transferred to hemodialysis, and their catheters were removed. Surgical exploration was performed in 4 patients. All patients were treated with corticosteroids, and three received tamofoxin which was discontinued in one patient after 2 months because of liver damage and in one patient because of allergic reaction. A female patient on tamofoxin received total parenteral nutrition for 2 months. She recovered well and died of other reasons 2 years later from severe heart failure after implantation of cardioverter defibrillator. She had enormously pronounced left ventricular hypertrophy. One male patient died two years after the diagnosis of SEP from severe disseminated atherosclerosis which demanded bilateral leg amputation. Other patients are still alive with the median follow-up of 9 months after the diagnosis of SEP.

Conclusions: We conclude that the prevalence of SEP increases in long-term CAPD patients. It is a life-threatening complication of CAPD, and should be considered in all patients who have been treated with PD longer than 6 years, in patients with ultrafiltration failure, and in patients with symptoms of bowel obstruction. The systemic nature of inflammation should be considered since it supports the role of immunosuppressive drugs in treating this severe condition.

O39

AUTOMATED PERITONEAL DIALYSIS IN PATIENTS WITH DOWN SYNDROME

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Objectives: Scarce information is available about renal failure and renal replacement therapy in patients with Down syndrome. They are often considered unfit for peritoneal dialysis (PD) mainly because of the lack of compliance and increased risk of peritonitis. We report our experience with two adult Down syndrome patients treated with chronic PD.

Methods: Two Down syndrome patients (1M/1F, aged 33 and 31 years) with end-stage renal disease, resulting from reflux nephropathy and chronic glomerulonephritis, were selected for treatment with peritoneal dialysis after thorough briefing with their parents (mothers) who were to follow the educational program and training for the application of the method. Male co-morbidities included hyperlipidemia, arterial hypertension, partial blindness and periododontal disease. The female patient suffered from hypothyroidism.

Results: In both patients a swan neck, double-cuff, Toronto-Western peritoneal catheter was inserted by laparotomy under general anesthesia. Post operative period followed without any complications and patients adapted easily to the new condition with their mothers’ attentive affection and care. Both of them had a significant residual renal function that allowed a four-week hating and adaptation period without PD treatment. After this period, 2 weeks of education on the method followed. Night APD was then started for 9-10 hours with 1.5L exchanges. Treatment continued uneventfully for 65 and 9 months respectively, without infectious complications and with monthly clinical and biochemical controls in our PD unit. Peritoneal dialysis efficiency was evaluated by PET test and weekly Kt/V only in the female patient as the male reacted negatively to the test application.

Conclusions: In patients with Down syndrome and end stage renal disease, peritoneal dialysis is a successful renal replacement option as far as there is a willing primer caregiver and a stable and supportive family environment. Automated peritoneal dialysis technique may protect caregivers from burnout syndrome and guarantee effective treatment and method survival.

O40

DEVELOPMENT OF A CAPD CATHETER WITH INFECTION PROOF EXIT SITE

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Objectives: The CAPD – continuous ambulatory peritoneal dialysis – is a therapy, which requires the passage of a lead through the skin. The danger of an exit site infection prevents the clinical application. The infection enters the body through the three-phase line, where air, implant, and tissue meet. A CAPD catheter is implanted in the abdominal wall in a tunnel, which runs parallel to the skin. In the middle the catheter is equipped with a cuff of inert textile fibers to allow an ingrowth of connective tissue. The part from this cuff
to the exit site is where an infection starts. A few days after implantation this part of the catheter is covered with a biofilm, which grows and persists in the wet environment full of nutrients. The objective is to stop the infection from entering the body.

Materials and Methods: In order to avoid an infection, the catheter is covered by a sleeve of a thin membrane, which is slowly pulled out of the body. This sleeve is on its outside covered with fibers for an ingrowth of cells. Pulling on the sleeve results in an outward movement of the three-phase line, which counteracts the inward movement of the microbes. The dry air does not permit the growth of a biofilm. The technical solution comprises an implant with a surrounding sleeve, which is a thin foldable membrane. One end it is partially implanted in the body and the other distal end is passing through the skin. An external mechanism provides a “growth” – by a soft pull the sleeve is slowly moved out of the body. A renewable air-implant interface is created.

The growth of a biofilm. The technical solution comprises an implant with a surrounding sleeve, which is a thin foldable membrane. One end it is partially implanted in the body and the other distal end is passing through the skin. An external mechanism provides a “growth” – by a soft pull the sleeve is slowly moved out of the body. A renewable air-implant interface is created.

Result: 14 catheters with an outer diameter of 5 mm have been implanted in three goats. Four silicone rubber catheters had no sleeve, six were equipped with a sleeve, though inactive, and four catheters had an active sleeve. While two silicone rubber catheters and two inactive catheters became infected and were explanted, all active catheters remained without any sign of infection 12 months postoperatively.

Conclusions: The proposed active catheter offers a new solution to the infection problem of CAPD catheters.

**O41**

**RANDOMIZED, PROSPECTIVE STUDY OF ZURAGEN™ VERSUS HEPARIN AS LOCKS FOR TUNNELED CENTRAL VENOUS CATHETERS FOR DIALYSIS (TFC/FCGCD)**

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Objectives: Zuragen™ solution is an experimental catheter lock comprised of 7% sodium citrate at pH 6.2, methylene blue and parabens, with antimicrobial efficacy against all planktonic bacteria and bacteria within biofilm. We performed a multcenter, randomized prospective clinical trial comparing Zuragen™ and heparin (5000 units per lumen) as catheter lock in ESRD patients with CVCD of various types and ages, to determine whether the new lock decreases the incidence of catheter related blood stream infection (CRBSI) while maintaining catheter patency.

Materials and Methods: Primary endpoint was the incidence of catheter related blood stream infection (definite CRBSI=concordant blood culture from the catheter and peripheral blood in subjects with fever) and secondary endpoints included incidence of suspected CRBSI (positive blood cultures) and catheter related patency failure (removal of catheter after a decrease in hydraulic conductance of at least 20%, after up to three interventions). Exclusion criteria included patients with active infection or receiving antibiotics within the prior two weeks. Twenty six sites enrolled 416 patients; all 407 treated patients were analyzed. All endpoint data have been adjudicated by an independent clinical endpoint committee.

Results: Patient-level incidences of combined definite/concordant CRBSI (62.7% relative reduction [RR]; p = 0.001) and adding suspected CRBSI (63.8% RR; p = 0.003) achieved statistical significance as did preplanned composite endpoints. There was no difference in average blood flow rate, adverse events, or hospitalizations between the two groups.

Conclusions: As a lock for CVCD, Zuragen™ may diminish the incidence of CRBSI while providing long-term patency, thus making the use of CVCD safer and more effective as blood access for hemodialysis.

**O42**

**NOSOCOMIAL INFECTIONS IN PATIENTS WITH TUNNELED HEMODIALYSIS CATHETERS**

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Objectives: The goal of the trial was to evaluate rates of catheter colonization and bloodstream infection with jugular catheterization compared with femoral catheterization in patients undergoing chronic hemodialysis therapy (TFC) and jugular catheters (TJC) were tunneled and used as a permanent vascular access.

Methods: We analyzed: time to colonization on removal of TJC or TFC (reported as per 1000 catheter days); rates of catheter-tip colonization, defined as cultures with 10 colony-forming units/mL from the catheter tip; and rates of catheter-related bloodstream infection, defined as colonization plus 1 peripheral blood culture yielding the same species within 48 hours of catheter removal.

Results: Characteristics of patients who received TFC or TJC were similar. 144 catheters were included in the analysis and were grouped by insertion site of the catheter: 1. TFC n=103; and 2. TJC, n= 41. Duration time of TFC was 9899 days, average 139 days, and for TJC was 10 719 days, average 428 days. There were no differences in rates of catheter-related infection between femoral and jugular groups (incidence per 1000 catheter-days, 4.1 vs. 2.8; p = 0.42). The risk of catheter-tip colonization was not statistically significant between the femoral and jugular groups (hazard ratio, 0.85 [95% confidence interval (CI), 0.62-1.16]; p = 0.31). There were no significant differences in the rate of malfunction in the femoral group as compared with the jugular group (46.6% vs. 44%; p = 0.16). Tunneled jugular catheters took longer to insert, had more failures, and required more crossover insertion to the other site.

Conclusions: The incidence of catheter colonization, catheter-related bloodstream infection, and thrombosis is similar between the jugular and femoral tunneled catheters in the patients on chronic hemodialysis program. This finding goes counter to the widely held belief that more infections are experienced with the femoral access site.
including 10% human plasma and 10 ng/ml LPS or control medium for 20 hours. THP-1 and HUVEC, as well as their derived microparticles, were analyzed by flow cytometry, whereas concentrations of TNF-α, IL-10, IL-6, IL-8 and IL-10 in the supernatant were analyzed with Lumimex System (BioRad).

**Results:** The analysis of the surface protein expression showed changes due to flow conditions and presence of LPS in both cell populations (CD54, CD142 for THP-1 and CD54, CD141, CD25E, CD62P for HUVEC). Analysis of supernatant showed increased levels of TNF-α caused both by flow and presence of LPS and a different pattern of interleukin concentration levels in comparison to the control.

**Conclusions:** Because of the higher similarity to the in vivo conditions and the proved importance of the presence of flow on the cell response, our method is a promising tool to simulate inflammation processes caused by Gram negative bacteria, like sepsis.

**O45**

**MICROFLUIDIC BIOCHIPS AS NEW SUPPORTS FOR IN VITRO BIOARTIFICIAL ORGANS AND TOXICITY STUDIES**

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**Objectives:** Current developments in the technological fields of tissue engineering, bioengineering, biomechanics, microfabrication and microfluidics have led to highly complex and pertinent new biochips for in vitro toxicity investigations. The purpose of the biochips is to mimic organ tissues in vitro in order to partially reduce the amount of in vivo testing. These biochips consist of microchambers containing engineered tissue and living cells cultures interconnected by a microfluidic network, which allows the control of microfluidic flows for dynamic cultures, continuous feeding of nutrients to cultured cells and waste removal. The biochips also allow the control of physiological contact times of diluted molecules with the tissues and cells. Cell biochips can be situated between in vitro Petri testing and in vivo testing. In this frame we present a biochip that can be applied to various cell types in order to be able to study toxicity on reconstructed tissues.

**Materials and Methods:** PDMS sheets were fabricated by micro molding. The biochips with a volume of 30 µL were fabricated by 2 PDMS sheets bonding. Demonstration of cell cultures was performed with human liver HepG2/C3A, MDCK and mouse pre-osteoblast MC3T3-E1 cell lines.

**Results:** Healthy culture conditions during 96 hours, including 72 hours of perfusion were demonstrated by cell proliferation (MDCK, HepG2, MC3T3) and basic metabolism activities. An example of chronic toxicity was performed by using ammonia chloride loadings that have reduced the proliferation of the basic metabolism activities. An example of chronic toxicity was performed by using ammonia chloride loadings that have reduced the proliferation of the basic metabolism activities.

**Conclusions:** The developed biochip was found to be suitable for various types of cell cultures. It successfully demonstrated its potential for toxicity studies. In future work, toxicity analysis will be further investigated on a wide range of xenobiotics.

**O46**

**EMBRYONIC STEM CELL CULTURE IN AN IN VIVO MIMICKING MEMBRANE-BASED MICRO-BIOREACTOR**

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**Objectives:** During early stage of in vivo development, embryonic stem cells (ESCs) undergo biological and morphological changes in dynamic microenvironment. Such a microenvironment facilitates intimate cell-to-cell contact and enrichment of various self-secreted factors. To develop an advanced tool for mechanistic understanding of such changes of ESCs in vivo, they were cultured in two-chambered membrane-based micro-bioreactors of two different heights (192 and 506 µm).

**Materials and Methods:** Micro-bioreactors were made of PDMS polymer using basic MEMS technology. SiO2 coated Polyester membrane (pore size = 0.4 µm, membrane area = 2.24 cm2) was placed in the middle of two chambers and the whole structure was bonded using plasma bonding. ESCs were cultured on the top face of the membrane in culture medium containing Knockout serum (GIBCO), which contains fewer extrinsic proteins than bovine serum. Culture medium of lower chamber was only changed to keep the ESCs microenvironment in the upper chamber undisturbed. As a control, a 6-well plate culture was carried out by changing the medium daily. Cells were cultured for five days in both culture systems. RT-PCR analysis was carried out for pluripotent genes (Oct4, Sox2, Nanog and Rex1) and for some self-secreted factors (Fgf4 and BMP4).

**Results:** The colonies of ESCs remained unmerged in both micro-bioreactors but their sizes were found larger in the bioreactor of higher height. Both bioreactors gave similar low glucose consumption and aerobic condition compared with the plate culture. Cell growth was higher in the bioreactors of higher height than that of lower height. In parallel, the expressions of pluripotent marker genes were down regulated in the bioreactor of lower height compared to that of higher height, although self-secreted factor genes were expressed similarly in both reactors.

**Conclusions:** Enhanced differentiated nature of ESCs in the bioreactors of lower height demonstrates the importance of proper design of artificial microenvironment for better in vivo mimicry. We further investigate whether the bioreactors having different sizes of microenvironments accelerate differentiation to some specific lineages.

**O47**

**EMBRYO CULTURE SYSTEM USING DYNAMIC MICROARRAY**

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**Objectives:** We have proposed and developed an automated embryo culture system, which can make an array of embryo, culture them to be blastocyst culture, manipulate the blastocyst for the quality control for reproductive technology. We previously proposed and experimentally developed a way to manipulate numerous particles in microfluidic device under precise fluidic control, which is named “dynamic microarray”. In this study, the dynamic microarray has been applied to the culture system for embryo manipulation.

**Materials and Methods:** We preliminary examined independently the basic three functions of trapping, culture, and release using a mouse embryo as a sample. 1 and 0.5 µL/min flow rates were used for trapping and perfusion culture, respectively. The embryos were observed morphologically by using time-laps microscope during the culture. When a blastocyst appeared, the laser was irradiated onto an aluminum pattern near the blastocyst to generate a bubble for blastocyst collection.

**Results:** The embryos are successfully trapped for each cage at 1 embryo/2.5 sec rate with the 1µL/min carrier flow rate. The embryo, which became blastocyst normally in the dynamic microarray, had no morphological difference compared with the cultured one by a conventional microdrop method. However, the rate of embryonic development using the device was lower than that using the conventional method. The blastocyst was successfully released from the cage by the microbubble.

**Conclusions:** We have proposed and experimentally tested the automated embryo culture system using the dynamic microarray. As a result of our preliminary testing, mouse embryos are successfully trapped, cultured, and collected, whereas the efficiency of the in-device embryo culture was less comparable than the conventional dish culture. The culture condition still needs optimization for embryos, however the concept of the embryo manipulation was proofed successfully.

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**CARDIAC ASSIST (CLINICAL)**

**O48**

**EARLY AND LATE OUTCOME OF 463 CONSECUTIVE PATIENTS TREATED WITH EXTRACORPOREAL MEMBRANE OXYGENATION FOR REFRACTORY POSTCARDIOTOMY CARDIOGENIC SHOCK (PCS)**

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**Objectives:** PCS occurs in 1-2% of cardiac surgery patients. Hospital and long-term results of 463 consecutive patients receiving ECMO implantation were analyzed.

**Methods:** Between 05/96 and 05/08 463 out of 40,538 patients (1.1%) undergoing cardiac surgery (76.9% elective, 23.6% urgent, 38.5% emergency) received perioperative ECMO support. Procedures were isolated CABG (37.4%), CABG+valve surgery (15.5%), valve surgery (16%), thoracic organ
transplantation (5.8%) and others (25.3%). Fifty-four preoperative, 26 intraoperative and 37 postoperative risk factors were evaluated by uni- and multivariate logistic regression analysis to identify risk factors and follow-up mortality. Cumulative survival was estimated by Kaplan-Meier analysis.

**Results:** Age was 63.5y, 71.5% were male, ejection fraction was 45.9±17.6%, logEuroScore was 21.6±20.7%. ECMO implantation was performed through thoracic (61.8%) or extrathoracic (38.2%) cannulation using femoral or axillary arterial and Femoral venous cannulation. Additional IABP support was employed in 74.1%. Mean drainage loss was 4.2 after 48h, 63.3% (293 pts.) were successfully weaned from ECMO and 24.8% (115 pts.) were discharged from the hospital. Mean duration of ECMO support was 3.28±2.85 days. Cerebrovascular events occurred in 17.5%, gastrointestinal complications in 18.8% and renal replacement therapy was indicated in 65.5%. Hospital mortality was 75.2%.

Significant pre- and procedural risk factors for hospital mortality were diabetes, preoperative renal insufficiency (>1.8mg/dl), body mass index (>30), logEuroScore (>20), first lactate in OR (>4mM) and paceremaker dependence of the op, while coronary artery disease was none. Cumulative survival was 17.6±1.8% after 6 months, 16.5±1.7% after one year and 13.7±1.7% after five years. Mean follow-up of all hospital survivors was 3.21y (0.0 - 10.3y). Out of the 115 discharged patients, 52 (45.2%) died during follow-up time.

**Conclusions:** Temporary ECMO support is an acceptable option for patients with PCS that otherwise would die and justified by good long-term survival of hospital survivors. However, because of high morbidity and mortality individual ECMO indication has to be made on the specific risk profile.

**O40 EARLY CLINICAL EXPERIENCE WITH PARTIAL SUPPORT PROVIDED BY THE SYNERGY DEVICE**

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**Objectives:** Left ventricular assist devices (VADs) are used in end-stage chronic heart failure (CHF), mainly in patients with (or near) cardiogenic shock. VAD use is restricted because they require a major surgery and are associated with major adverse events. Smaller, less invasive devices could expand VAD use to less sick patients. However, smaller devices pump less blood (partial support), which raises 2 important questions, related to: 1) appropriate selection criteria for patients who would benefit from partial support, 2) confirmation that partial support provides long-term benefits.

**Methods:** The SynergyTM pump (size of a AA battery, 25 grams) pumps 2.5-3.0 L/min, is implanted (off pump) with a mini-thoracotomy and is positioned subcutaneously in a right subclavian pacemaker-like pocket. The inflow graft inserts into the left atrium; the outflow connects to the right subclavian artery.

**Results:** 15 patients (12 males), with baseline EF 19% (10-32%), arterial pressure 73 (63-81) mmHg, PCWP 38 (33-53) mmHg and CO 3.7 (2.5-4.8 L/min) were implanted with a Synergy device. Duration of support averaged 83 (8-213) days. 87% (13/15) of patients were alive at 3 months; 9 were bridged to transplant. 6 patients had protocol-driven follow-up right heart catheterization; at 9.5 (5-19) weeks, increases in arterial pressure (71±4 vs 83±8, p=0.05) and cardiac output (4.0±0.8 vs 5.2±5.2, p=0.005) with large reductions in capillary wedge pressure (30±5 vs 15±4, p=0.001) were observed. Peak VO2 increased by 3.0±0.5 mL/kg/min (10.7±2.2 vs 13.7±2.2, p=0.008).

**Conclusions:** Partial support provided by the Synergy device yielded good clinical outcomes in patients. Such support interrupts the progressive hemodynamic deterioration typical of late stage CHF. This device has the potential to expand use of VADs to a large population of patients with severe medically refractory CHF not currently sick enough to justify the risks associated with implantation of currently available full support VADs.
OS2

ASSESSMENT OF ECG-TRIGGERED MUSCULAR COUNTERPULSATION FOR HEMODYNAMIC IMPROVEMENT OF CARDIAC FUNCTION
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Objectives: ECG-triggered peripheral muscular counterpulsation (MCP) was proposed as a new non-invasive technique for the treatment of acute cardiac failure in coronary artery disease (CAD). Study purpose: 1) evaluate safety and efficiency of MCP; 2) determine its hemodynamic effect on cardiac function.

Materials and Methods: In 16 patients (7 no CAD and 9 CAD) MCP was performed on calves, thighs and lower abdomen for 3 minutes each at low (<15 V) and high (15–25 V) amplitudes. ECG-triggering was used to synchronize stimulation during early diastole. LV function was assessed by the conductance method using simultaneous LV pressure-volume loops. Heart rate, mean aortic, LV end-diastolic and end-systolic pressures, LV volumes were measured. LV ejection fraction, dp/dt, cardiac output, systemic vascular resistance and cardiac efficiency were calculated.

Results: MCP was associated with an increase in cardiac output at all 3 stimulation sites. In CAD patients, there was 22% decrease in peripheral resistance, 12% increase in cardiac index, 18% diminution of end-diastolic pressure, and 16% reduction in stroke work. Hemodynamic effects were less marked in control patients. There were no complications but 5 of 16 patients reported some itching sensations during stimulation.

Conclusions: MCP is safe and efficient for improving cardiac function in patients with CAD. The technique is completely non-invasive and simple to use with a portable stimulation device. Best effects of MCP are obtained in CAD patients with abdominal or thigh stimulation.

OS3

RISK ASSESSMENT OF PATIENTS SUFFERING FROM ACUTE MYOCARDIAL INFARCTION COMPLICATED BY CARDIogenic SHOCK RECEIVING EMERGENCY CORONARY ARTERY BYPASS GRAFTING
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Objectives: Cardiogenic shock (CS) is the most common cause of death in patients hospitalized with acute myocardial infarction (AMI). Aim of this study was to evaluate risk factors for mortality of patients suffering from AMI+CS undergoing emergency coronary artery bypass graft surgery (CABG).

Methods: Between 01/2000 and 12/2007 302 pts (68.74 years; 76.2% male; 42.5% STEMI, 97.6% multivessel-disease, 47.7% left main disease) underwent CABG. Risk factors for mortality were evaluated by uni- and multivariate logistic regression models. Cumulative survival was estimated by Kaplan-Meier methods.

Results: Hospital mortality was 37.1% (112 pts.). Univariate preoperative risk factors for hospital mortality were preop renal dysfunction [Odds ratio (OR) 2.3, p=0.004], EF <30% (OR 1.7, p=0.032), logistic EuroSCORE >20 (OR 19.2, p<0.001), STEMI (OR 1.83, p=0.013), preoperative lactate level >4mm (OR 3.58, p=0.001) while renal dysfunction, EuroSCORE >20 and lactate level >4mm were also significant independent risk factors. Preoperative use of IABP was beneficial. Hospital mortality was increased in CPB time >100 min (OR 1.77, p=0.018), delayed IABP (OR 1.98, p=0.013) and ECMO support (OR 4.15, p=0.001), while LIMA use was protective (OR 0.29, p<0.001). During postoperative course CK-MB fraction >120U/l on POD1 (OR 3.88, p<0.001), high blood loss (OR 2.04, p=0.007) and all cardiovascual and cardiac events and acute renal failure (OR 5.92, p<0.001) were risk factors for mortality. Cumulative survival was 50.7±2.9% after 1 year, 46.1±3.0% after 3 years and 38.2±3.3% after 5 years. Risk factors for follow-up mortality were diabetes (OR 1.95, p=0.034), COPD (OR 4.22, p=0.002) and renal dysfunction (OR 2.52, p=0.027). Complete revascularisation was protective (OR 0.45, p=0.026). There was a 1-year/3-year-freedom from AMI of 98.2±1.0/80.8±3.1%, and freedom of repeat revascularization of 95.1±1.7/89.1±2.8% respectively.

Conclusions: Mortality of emergency CABG in AMI+CS is strongly dependent on preoperative patient status and cardiac and extracardiac morbidity. Good long-term outcome of hospital survivors demonstrates the benefit of CABG in AMI+CS patients with otherwise bad prognosis. Early IABP implantation, LIMA use and a complete revascularization improve the prognosis.

ARTIFICIAL KIDNEY (CLINICAL)

OS4

CHANGES IN INDICATIONS FOR RENAL REPLACEMENT THERAPY IN CARDIOLOGY WARDS OVER THE LAST 6 YEARS
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Objectives: The union of cardiology and nephrology is manifested by increased number of invasive evaluation of coronary artery disease procedures performed in patients with the end-stage renal disease, as well as by increased number of dialysis treatments in patients with cardiac disease with or without concomitant renal failure.

Materials and Methods: We prospectively followed-up all patients who required renal replacement therapy during hospitalization in the cardiology department. Age, gender, indication for dialysis, SOFA score, method of renal replacement therapy, vascular access, number of treatments and outcome were recorded.

Results: From January 2003 to December 2008, 180 patients (22.2% women) age ranging from 22 to 85 years, required dialysis therapy during their hospitalization in the Cardiology department. 53.3% were admitted to the coronary unit, 46.7% stayed in other Cardiology wards. 58.3% of patients had end-stage renal disease, while 23.3% had refractory state of heart failure. Other indications included hyperkalemia and acute renal failure. SOFA score ranged from 4 to 21 (average 8.58). Endovascular catheter was used for vascular access in 53.8% and arteriovenous fistula in 46.1% of patients. Hemodialysis was performed in 24.4%, SCUF in 11.1%, while 64.5% received treatment with different continuous renal replacement therapies. Lethal outcome occurred in 26.67% of patients. Changes during the observed period included increase in number of patients per year, higher SOFA score indicating more complicating patients, increase in number of procedures per patient, increased percentage of procedures performed in the Coronary unit, increased number of patients with dilatative cardiomyopathy and refractory heart failure as well as patients with heart transplant.

Conclusions: A steady increase in the number of patients who require renal replacement therapy during hospitalization in the Cardiology ward was observed. The number of invasive procedures performed in patients with end-stage renal disease and dialysis treatments to support the failing heart without evidence of renal injury had both increased. The convergence of clinical cardiology and nephrology is increasing and requires stronger efforts of both subspecialties in order to achieve optimal treatment results.

OS5

IONIC DIALYSANCE AND UREA CLEARANCE IN ON-LINE HEMODIALFILTRATION
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Objectives: To establish whether ionic dialysance (D) calculated from dialysate conductivity measurements is a valid surrogate for urea clearance in post dilution on-line hemodiafiltration (OL-HDF) with the AK200 ultra S (Gambro®, Sweden) dialysis monitor, equipped with automatic D measurements.

Methods: Records of D during OL-HDF were compared to paired measurements of instantaneous urea clearance (UK) calculated from both blood and dialysate side. A mean of 3 measurements per dialysis session were performed using different dialysate, blood and reinfusion flow rates. Seven patients were dialyzed on double tunneled catheters and 9 on arteriovenous access.

Results: Sixty-four records of D have been performed during 18 sessions. Five pairs of measurements were not included into the analysis because of unexpected values of UK. UK from blood and dialysate side were not statistically different (-0.3 ± 31 mL/min, paired t test). The mean of both
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measurements were then used for subsequent statistical analysis. The mean values of D and UK were respectively equal to 206 ± 22 mL/min and 235 ± 22 mL/min with a mean difference of paired data of -28 ± 27 mL/min (p = 0.03). The two parameters were linearly correlated (r = 0.27, p = 0.03).

Discussion: The difference observed between UK and D could be explained by the recirculation which is taken into account by D measurement but not by the instantaneous measurement of UK. Central venous catheters are expected to induce more access recirculation whereas dialysis efficiency for dialysis sessions on arteriovenous access is decreased by cardiopulmonary recirculation. Both phenomena lessen the dialysis efficiency and could explain the discrepancy observed between D and UK.

Conclusions: Our study evidenced a 10% difference between D and UK during OL-HDF, probably related to recirculation.

**O56**

**WHAT IS THE BEST DIALYSIS STRATEGY TO TREAT BURN-INJURED PATIENTS WITH ELEVATED IODINE BLOOD LEVELS?**

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Objectives: In burn-injured patients, the topical antimicrobial agent povidone-iodine (PI) is widely and successfully used to avoid infection. However, long-term application on open wounds of PI, which is primarily excreted by kidneys and captured by thyroid glands, is known to induce toxicity and acute kidney injury (AKI). As little is known about the kinetics of iodine, the present study aimed to determine iodine kinetic behavior during hemodialysis and define the best hemodialysis strategy for its removal.

Materials and Methods: This study included 2 patients admitted to the Burn Unit with AKI and elevated iodine levels (93.6 and 81.2 mg/L, respectively). Both patients were started on continuous dialysis using the Genius® dialysis system with blood flows of 150 and 120 ml/min, respectively. In the first patient, blood was sampled from the arterial and venous bloodline at different time points during the first 7 hours of dialysis. Patient 2 was treated during 44 hours while arterial and venous blood samples were taken every 6 hours. Blood samples were analyzed for total iodine. These concentrations were further used to calculate dialyzer clearance and to fit the kinetic parameters of a compartmental distribution model for iodine.

Results: During the first 7h of dialysis in patient 1, iodine was found distributed in a single compartment of 26±5 L. However, the 44h data of patient 2 unravelled that after 7h a 2nd compartment comes into play. In patient 2, iodine was distributed in 2 volumes of 19±1 L and 38±1 L, with a slow intercompartmental transport (55±4 mL/min). Based on simulations, those findings imply that slow continuous dialysis is preferable above an intermittent dialysis regimen, even when using higher blood flows of 300 mL/min.

Conclusions: If burn-injured patients with elevated iodine levels are exposed to PI adsorption and develop AKI, the best choice is to perform slow continuous dialysis.

**O57**

**EFFECT OF DIALYSIS FLUID BICARBONATE CONCENTRATION ON ACID-BASE BALANCE IN HEMODIAFILTERATION: IN VITRO AND MATHEMATICAL MODEL**

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Objectives: Blood acidosis is corrected in hemodialysis (HD) by bicarbonate buffer (HCO3- ) in the dialysate fluid; in hemodiafiltration (HDF), HCO3- kinetics might be disturbed by the addition of convection. To closely analyze the effects of each technique on HCO3- kinetics, we propose to develop both in vitro and mathematical models.

Materials and Methods: The patient is represented by a 2L bag of fresh bovine blood which circulates through a 0.6m² hemodialyser. Nine tests are carried out at a blood flow of 200ml/min: three in HD, six in online HDF with different filtration rate (Qf) or with HDF 30/50 or 50/30. The HDF dialysis fluid concentration (Cd) is set at 28, 32 or 40mmol/L. The in vitro HCO3- blood concentrations are compared with theoretical ones calculated by a mathematical model using mass balance equations for the patient and local mass transfer in the dialyser.

Results: Good agreement between theoretical and experimental concentrations is obtained. In HDF, HCO3- mass transfer occurs from the blood to the dialyzer because of large ultrafiltration. The gain of HCO3- for the patient is achieved by the reinjection. The empirical correlation for final HCO3- plasma concentration (Cf) in function of the initial one (Ci) can be given by: Cf = 0.2Cf + Ci (Cd = 6.4).

Conclusions: Our model seems to adequately reproduce the HCO3- kinetics. It can be used to compare efficiencies of various HDF strategies. The next step will be to compare it with in vivo data obtained on dialysed patients.

**O58**

**BRAIN NATRIURETIC PEPTIDE IN HEMODIALYSIS PATIENTS: PROGNOSTIC VALUE OF BASELINE MEASUREMENT FOR ALL-CAUSE AND CARDIOVASCULAR MORTALITY**

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Objectives: Brain natriuretic peptide (BNP) is a predictor of mortality in non-renal cardiovascular (CV) diseases but its value in hemodialysis (HD) patients is still a matter of debate. The aim of this observational and prospective study was to evaluate the role of BNP as a prognostic factor in terms of all-cause and CV mortality in HD patients treated in a single centre.

Methods: We measured global concentration of BNP in 235 prevalent HD patients (mean age at beginning of HD 48.8±14.95 years, mean hemodialysis vintage 99.2±60.77 months) to examine the two-year all-cause and CV mortality associated with baseline BNP concentrations. Sensitivity, specificity and cut-off levels for pre-HD BNP as predictor of all-cause and CV mortality were analyzed by means of receiver operating characteristic (ROC) curve.

Results: The mean pre-HD BNP value was 1621.25±2995.16 pg/mL (103.1 - 29190). During the 2-year follow-up, 35 out of 125 patients (28%) died, most from CV diseases (85.7%). Patients who died of CV causes had higher levels of BNP (3736.6±6239.16 vs. 1110.6±1132.13; p=0.0002), as well as patients who died of all causes (2947.8±5178.76 vs. 1105.3±1136.64; p=0.0017). There was a positive correlation between BNP and systolic blood pressure (SBP) (r=0.32, p=0.0006), pulse pressure (PP) (r=0.35, p=0.0000), left ventricular mass index (LVMi) (r=0.35, p=0.0002), and inverse correlation between BNP and hemoglobin (r=-0.32, p=0.0001), creatinine (r=-0.28, p=0.0013) and ejection fraction (r=-0.2, p=0.041). Patients with BNP>1200 pg/mL had significantly higher SBP (145.3±38.33 vs 128.7±20.42mmHg, p=0.000) PP (60.53±16.03 vs 49.39±11.32mmHg, p=0.000), C-reactive protein (12.13±11.28 vs 7.86±6.44mg/L, p=0.007), LVMi (160.91±22.76 vs 127.27±39.28, p=0.000mg/m²) and significantly lower hemoglobin (103.7±9.92 vs 110.91±9.40g/L, p=0.000) than those with BNP<1200 pg/mL. The cut-off point for BNP as predictor of all-cause and CV mortality was analyzed by median of receiver operating characteristic (ROC) curve.

Conclusion: The difference observed between BNP and hemoglobin (r=-0.32, p=0.0001), creatinine (r=-0.28, p=0.0013) and ejection fraction (r=-0.2, p=0.041) was statistically significant. Patients with BNP>1200 pg/mL, at increased risk of cardiac events, may improve their prognosis.

**O59**

**QUALITY OF LIFE AND INTRADIALYTIC HYPOTENSION**

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Objectives: To assess the impact of intradialytic hypotension (IDH) on the quality of life (QoL) in patients on maintenance dialysis.

Methods: The SF-36 (Sort Form – 36 Health Survey) was validated in 136 patients. The socio-demographic data, dialysis practice variables, laboratory values and comorbidities were noted. Nutritional and inflammatory state were assessed by subjective global assessment (SGA), body-mass index (BMI) and malnutrition - inflammation score (MIS) - determined by the method of Kalantar et al. Univariate and multivariate regression analysis were performed.

Results: We found that female gender, older age, lower social status, sleep disturbance, poor family support and presence of diabetes are associated with lower QoL scores. No significant association was found for the Hg levels, probably due to the optimal values found in the vast majority of our patients. The strong associations between all the physical and mental health concepts and the albumin level didn’t hold after adjusting for the confounding factors. The associations with BMI and CRP were inverse and insignificant. The presence of Hepatitis C had no influence on the QoL, as the dialysis timing...
and vintage, but the low dialysis adequacy independently worsened the Mental component score. The IDH correlated inversely and independently with all SF-36 dimensions except for the social functioning scale. The patients having IDH had significantly lower values for k/t and albumin, and significantly higher values for CRP. UF rates as percentage of dry body weight per hour, rates of thromboses of fistulas and chronic interdialytic hypotension presence. In the multiple regression model, the age, family support, sleep disturbance, creatinine, SGA, DM and IDH were the strongest independent predictive markers for the Physical component score. For the Mental component score age, family support, adequacy, creatinin, MIS and IDH were the strongest predictors.

**Conclusions:** The Quality of Life is influenced by the Intradialitic hypotension to a remarkable extent. Preventing the frequency of the hypotonic episodes the QoL should be improved.

### MICROENCAPSULATION AND ARTIFICIAL ORGANS

**O60**

THE ENCAPSULATION OF MONOCYTES INTO POLYLECTROLYTE CAPSULES FOR THE TREATMENT OF SEPTEPSIS

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**Objectives:** Cellular entrapment within polymer microspheres has been substituted to extensive research since the 1980s. The aim of this work was the encapsulation of monocytes, thereby entrappling cellular mass inside polymer capsules of ideally 100 µm diameter for the use in an extracorporeal blood purification system, taking regulatory influence on inflammation-induced cytokine levels in patient's blood.

**Materials and Methods:** A protocol for the production and subsequent characterization of monocytes containing alginate beads, using the Inotech IESO R Encapsulator, surrounded by a polyelectrolyte complex, was developed. Characterization of produced microcapsules included investigation of size distribution, capsule thickness, stability testing as well as permeability studies. Polymers surrounding an alginate core bead were selected such as to guarantee a semipermeable membrane, regulating diffusion of molecules of defined molecular weight. The efficacy of Alginate-chitosan-alginate (ACA) microcapsules, allowing monocyte survival of the encapsulation procedure itself as well as subsequent intracapsular proliferation was established. Functional analysis of encapsulated cells included monitoring protein secretion and metabolic activity. Relative quantification of intracapsular proliferation was required to draw comparison between confined and unconfined cellular growth. Methods of cryopreservation of entrapped cells were examined with regard to the effect on capsule integrity and cell viability.

**Results:** Successful encapsulation of monocytes into fully characterized ACA microparticles was achieved without negatively impacting cell functionality. Conclusions: Entrapment of monocytes into ACA microspheres presents the first step towards the capsules incorporation in blood purification circuits, with particular focus on the regulation of the concentration of cytokine levels in patient's blood. Hence, a possibility could be created for interference with the uncontrolled patient inflammatory response during sepsis.

**O61**

A CLINICAL SCALE BIOREACTOR FOR EXTRACORPOREAL BIOARTIFICIAL LIVER MACHINE USING LIVER-DERIVED HUMAN CELLS

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**Objectives:** To develop a bioartificial liver (BAL) support system on the clinical scale, as a therapeutic option for the survival of patients with acute liver failure. Materials and Methods: Our bioartificial liver machine is an extracorporeal device combining a biological component (liver derived cell line) in an artificial component (a fluidized bed chamber). For developing the device cells are grown to performance competence using 3-D culture of cells encapsulated in alginate beads. Subsequently, alginate beads are fluidized with the patient’s plasma in an extracorporeal circuit to supplement liver function. Large scale monolayer culture using a 25-stack Cell Cube device (Corning) supplied cells for encapsulation. The Jetcutter encapsulator (GeniaLab) was used for encapsulation of cells 1.5-gal with target alginate diameter of ~400µm and volume of 1.5 x 10^6/mL. Results and Conclusions: A protocol for the production and subsequent characterization of monocytes containing alginate beads to a cell density of >6 x 10^9/mL, and a total number of 5.25x10^10 cells, was obtained by day 13. Cell viability was maintained >95% throughout. Analysis of the media showed continuous glucose consumption by the cells, indicating active metabolic function, with a gradual lactate accumulation over time. A progressive increase in oxygen consumption and continued synthesis and secretion of alpha-fetoprotein, a liver specific protein, demonstrated useful function. Moreover these functions are maintained in acute liver failure plasma, and detoxification, as exemplified by bilirubin conjugation, occurs at a rate of 100mg/1x10^10 cells/day.

**Conclusions:** We have developed a BAL suitable for clinical use, able to house 7 x 10^10 cells in a volume of ~1300mL. The degree of function, e.g. bilirubin conjugation is suitable even for deeply jaundiced patients. BAL efficacy will now be tested in a porcine model of acute liver failure, using 20kg pigs and ~2 x 10^10 cells.

### ROTARY PUMPS: MONITORING THE PUMP, THE PATIENT AND THE HEART

**O63**

CONTINUOUS MONITORING OF CARDIAC CONTRACTILITY FOR ROTARY BLOOD PUMP RECIPIENTS: COMPARISON WITH CLASSICAL CONTRACTILITY PARAMETERS

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**Objectives:** Nowadays, rotary blood pumps (RBPs) are used also as a Bridge to Recovery (BTR) and for Destination Therapy (DT). In these applications it is particularly important to know the remaining cardiac contractility. Previously we presented a method to determine residual ventricular contractility from pump data only. Here we present the comparison with classical contractility indices.
Materials and Methods: The pump flow derived contractility index (IQ) was defined as the slope of the linear relationship between the maximal derivative of pump flow (dQ/dtmax) and the peak-to-peak value of pump flow (QP2P) during small speed variations. In vivo, IQ was compared with other classical pressure-volume derived indices (IPM= dP/dt end-diastolic volume; ISW= stroke work vs. end-diastolic volume). All indices were evaluated in 7 acute sheep experiments using a MicroMed VAD® at isotropic interventions. In clinical data from MicroMed VAD® recipients, IQ was evaluated and compared with other simple clinical flow parameters, QP2P and pulsatility index (PI = QP2P divided by mean pump flow).

Results: Linearity of the dQ/dt max vs. IQ relationship showed a linear correlation in both animal experiments and clinical data. In vivo, IQ was found to be 15.3±4.0 in the control condition, 9.3±3.9 for reduced contractility, and 15.9±2.5 for increased contractility (compared to IPM= 13.4±4.5, 6.5±3.1, 13.6±9.1; ISW= 38.3±12.3, 22.8±11.3, 54.2±38.6). In clinical data, IQ was shown speed-independent, but both QP2P and PI did highly vary with speed.

Conclusions: IQ is sensitive to change of cardiac contractility in a manner similar to other classical indices. This index is easy for cardiac contractility assessment in patients without additional measurement. This may give new perspective for improved diagnostics in Bridge to Recovery and Destination Therapy.

Mathematical Modeling for Artificial Organs

O64 MATHMETICAL MODELLING IN RENAL REPLACEMENT THERAPY
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Mathematical modeling (MM) has probably been more widely applied in the field of Renal Replacement Therapy (RRT) than in many other medical disciplines, although its aims here were the same as elsewhere – to increase understanding of processes which are taking place both in the patient’s organism and in the therapeutic device, to facilitate and support decision making in therapy planning and administration, and in some cases even direct on-line control of some part of the treatment procedure. Very varied methodologies have been used in MM in RRT, ranging from classical compartmental models with easy physiological interpretation up to the newer more abstract approaches like fuzzy logic, neural networks or fractal analysis. Some models become very well established and widely used – the Urea Kinetic Model used in the RRT centres are probably the most reliable. One additional functional block is the Three-Pore Model in peritoneal dialysis. The most widespread form of peritoneal dialysis, the CAPD, was even first modelled before it was attempted clinically. Some models in other areas of RRT, albeit badly needed, are still not good enough to guarantee clinical benefits when practically used, such as sodium and water kinetics in ultrafiltration control or models of thermal balance during hemodialysis. Rather specific class of MM in RRT are models created as a decision-supporting tool in drug dosage (systemic anticoagulation with heparin or regional citrate anticoagulation, anemia treatment with erythropoiesis stimulating agents). Although they are usually received a bit stand-offish, their application may significantly improve treatment outcome and may also make the treatment more economical (optimized anaemia treatment). Significant economical potential may also have the rather easy to construct but underutilised epidemiological models or RRT needs (e.g. prediction of prevalent patient counts, number of nephrologists, dialysis centres or places needed in a certain region).

The presentation will provide principles of different models in RRT and whenever possible also examples of their implementation outcome.

O65 MATHMETICAL AND COMPUTER MODELLING OF PERITONEAL DIALYSIS
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Peritoneal dialysis (PD) utilizes a living system of perfused tissues and unphysiologically high osmotic pressure to remove excess water and waste product from the body. Fluid and solute transfer during PD involves at least three different types of transport barriers: 1) cell layers, as endothelium, 2) interstitium, and 3) cells building a tissue, as muscle cells of the abdominal wall and gastrointestinal tract. Osmotically-driven ultrafiltration is a non-linear process that is difficult for mathematical modelling. Standard models for the analysis and simulation of the peritoneal transport, as membrane and three-pore models, apply a simplified description of the transport system as a single barrier between blood and dialysis fluid, and do not allow for separation of the contribution of different types of the transport barriers and the real geometry and size of the peritoneal transport system. Nevertheless, these models proved useful for the analysis of clinical and experimental studies and helped to reveal and explain the role of aquaporins in PD.

The distributed model allows for the combination of the physiology of fluid and solute transport across the capillary wall and across the tissue/interstitium with clinically relevant phenomena during PD. In particular, it allows for the calculation of the net transport parameters for the solute and fluid exchange between blood and dialysis fluid (diffusive mass transport coefficients, hydraulic conductivity, reflection coefficient, effective peritoneal blood flow) from the local transport parameters for the capillary wall and interstitium that are derived from physiological experiments. The model provides also information about spatial distributions of solute concentration and hydrostatic pressure in the tissue and therefore yields estimations of penetration depth for solute and fluid in the tissue.

The recent developments in the applications of distributed modeling for fluid transport, including peritoneal absorption into the tissue and glucose-induced osmotic ultrafiltration, open the way to physiology-based modelling of the transport processes during PD.

O66 DEVELOPMENT OF A MODELLING PLATFORM. THE CIRCULATORY MODEL: A TOOL FOR RESEARCH AND EDUCATION IN CARDIO-VASCULAR PATHO-PHYSIOLOGY
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Objectives: The aim of this work is the development of a modelling platform with hybrid capabilities: that is to say, its structure can be modified according to the experimental needs merging, if necessary, numerical models and physical devices or models that can be indifferentily hydraulic or electrical. The numerical circulatory model is an important part of the platform. It can be applied as a fully numerical model or as a part of a hybrid system.

Materials and Methods: The lumped parameter circulatory model consists of five functional blocks: left and right hearts, systemic, pulmonary and coronary membra and three pore models, apply a simplified description of the transport system as a single barrier between blood and dialysis fluid, and do not allow for separation of the contribution of different types of the transport barriers and the real geometry and size of the peritoneal transport system.

The results show that the numerical model is able to reproduce correctly patho-physiological conditions including a caval occlusion manoeuvre. In the hybrid version, the model is able to exchange data with the physical section of the circulation and is able to react to the action of LVAD assistance.

Conclusions: The model reproduces patho-physiological circulatory conditions and, in the hybrid mode, is able to react to mechanical heart assistance action. The applications shown here point out the possibilities of the model as a tool for research and education.

O67 VIRTUAL RESPIRATORY SYSTEM AS A TOOL IN RESPIRATORY SUPPORT INVESTIGATIONS
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Objectives: Although respiratory support (SUP) as such is not any complex scientific problem, SUP efficacy as well as health hazard related to SUP are problems very difficult to analyze because of significant nonlinearities in the respiratory system (RS) and several interactions between the cardiovascular system (CS) and RS. As those nonlinearities and interactions work one against the others, only such complex models as virtual RS can make it possible to...
analyze in detail the resultant effect of SUP on oxygen delivery and carbon dioxide removal with the smallest risk of lungs injury.

Methods: In general, there are two ways of the virtual RS use: (a) a virtual population is created by means of parameters randomly deviating and a problem (e.g. method sensitivity) is tested; (b) a standard virtual patient is treated with various methods analyzed comparatively. Analysis of contradictory effects of positive airway pressure on nonlinear airway resistance and lungs compliance suggests that, depending on obstruction disease severity, the same support may be profitable or not. Analysis of mechanical interaction between RS and CS, including gravity influence on individual regional toxicants and inpved biocompatibility. In addition to standard dialysis (MRS) and CS (MCS). Influence of the interaction on blood oxygenation requires an additional model of gas transfer and exchange (GTE). There are several ensembles composed of MRS and MCS and/or GTE in the literature.

Results: In the first part new membrane concepts combining different separation principles and functions to further increase removal capacity in dialysis applications will be presented. Integration of measurement functions leads to multifunctional devices allowing to reduce complexity and improve handleability. In the second part new membrane characterization studies will be presented. This unique membrane development (High-Cut-Off membrane) gives access to a whole group of new extracorporeal therapies. One example are patients with multiple myeloma suffering from elevated serum concentrations of monoclonal free light chains (FLCs), which can result in irreversible renal failure. In vitro and in vivo results using a High-Cut-Off membrane will be presented. Kazupa and lambda FLC sieving coefficient and clearance were studied in vitro. The technology platform for modern dialysis membranes based on synthetic polymers allows to tailor separation characteristics to the medical need. New membranes have the potential to further improve clinical outcome.

Conclusions: Complex models, such as virtual lungs, may be the best tool for analysis of many sophisticated phenomena related to respiratory support.

New Membranes for (Bio)Artificial Organs and Tissue Engineering

O68
NOVEL MEMBRANE CONCEPTS PAVING THE ROAD FOR IMPROVED AND NEW EXTRACORPOREAL THERAPIES
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Objectives: Today, dialysis membranes are highly engineered separation devices. However, there is a continuous request for increased removal rates of uremic toxicants and improved biocompatibility. In addition to standard dialysis membranes there is a need for more advanced / open membranes that allow effective removal of substances in the molecular weight range between 25 and 50 kDa (middle molecular weight substances).

Results: In the first part new membrane concepts combining different separation principles and functions to further increase removal capacity in dialysis applications will be presented. Integration of measurement functions leads to multifunctional devices allowing to reduce complexity and improve handleability. In the second part new membrane characterization studies will be presented. This unique membrane development (High-Cut-Off membrane) gives access to a whole group of new extracorporeal therapies. One example are patients with multiple myeloma suffering from elevated serum concentrations of monoclonal free light chains (FLCs), which can result in irreversible renal failure. In vitro and in vivo results using a High-Cut-Off membrane will be presented. Kazupa and lambda FLC sieving coefficient and clearance were studied in vitro. The technology platform for modern dialysis membranes based on synthetic polymers allows to tailor separation characteristics to the medical need. New membranes have the potential to further improve clinical outcome.

Conclusions: Complex models, such as virtual lungs, may be the best tool for analysis of many sophisticated phenomena related to respiratory support.

O69
MIXED MATRIX MEMBRANES FOR EXTRACORPOREAL BLOOD PURIFICATION
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Objectives: Therapy during renal failure requires the ongoing treatment of patients with hemodialysis. In the hemodialysis treatment, the blood stream containing uremic toxic compounds is dialyzed against a countercurrent flow of dialysate separated by a semi-permeable membrane. The dialysate is proportioned in each hemodialysis unit and is inevitably contaminated with bacterial substances and endotoxin. This issue, of bacterial contamination of dialysate, still represents a challenge for the attending nephrologists despite the availability of elaborate water treatment facilities. Investigations in dialysis centers revealed that a high percentage of the tested centers were using dialysis water and dialysis fluid exhibiting microbial contamination at levels above the standards set by the Association for the Advancement of Medical Instrumentation (AAMI). This presentation focuses on the removal of endotoxins by integration of adsorption properties and high flux membranes.

Materials and Methods: Activated carbon (AC) and ion-exchange resins (IERs) are embedded in porous cellulose acetate membranes, the so-called Mixed Matrix Membranes (MMM). The membranes are prepared by a vapor-induced phase separation process followed by immersion precipitation. The endotoxin removal performance was evaluated in both dialysis and dead-end filtration mode.

Results: The vapor-induced phase separation membrane consists of a highly porous layer that forms a porous phase in the MMM substructure. The endotoxin adsorption scales with the particle load in the MMM where the adsorption on AC is 50% higher than the adsorption on IERs. Increasing the ionic strength of the endotoxin solution favors the binding via hydrophobic interactions with the AC-particles. Adsorption experiments proved that back diffusion of endotoxin can be avoided by adsorption.

Conclusions: The MMM concept offers the possibility to capture endotoxins during hemodialysis or hemofiltration treatment. Co-casting of a particle-free silicon layer prevents particle loss and offers new possibilities for the optimization of porosity and biocompatibility. The MMM concept allows endotoxin removal and avoids endotoxin back transport by diffusion, convection and adsorption in one single process step.

O70
DEVELOPMENT OF MEMBRANE BIOHYBRID SYSTEMS FOR LIVER AND NEURONAL TISSUE ENGINEERING
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Materials and Methods: Activated carbon (AC) and ion-exchange resins (IERs) are embedded in porous cellulose acetate membranes, the so-called Mixed Matrix Membranes (MMM). The membranes are prepared by a vapor-induced phase separation process followed by immersion precipitation. The endotoxin removal performance was evaluated in both dialysis and dead-end filtration mode.

Results: The vapor-induced phase separation membrane consists of a highly porous layer that forms a porous phase in the MMM substructure. The endotoxin adsorption scales with the particle load in the MMM where the adsorption on AC is 50% higher than the adsorption on IERs. Increasing the ionic strength of the endotoxin solution favors the binding via hydrophobic interactions with the AC-particles. Adsorption experiments proved that back diffusion of endotoxin can be avoided by adsorption.

Conclusions: The MMM concept offers the possibility to capture endotoxins during hemodialysis or hemofiltration treatment. Co-casting of a particle-free silicon layer prevents particle loss and offers new possibilities for the optimization of porosity and biocompatibility. The MMM concept allows endotoxin removal and avoids endotoxin back transport by diffusion, convection and adsorption in one single process step.

Tissue reconstruction is still one field of important research, since the goal of producing perfect artificial tissue has not been achieved. The tissue engineering involves the in vitro seeding and attachment of human cells onto a nonwoven mesh-like layer that forms a continuous support for the specific tissue while secreting the extracellular matrix components, required to create the tissue. Therefore, the choice of material is crucial to enable the cells to behave in the required manner to produce specific tissues and organs. Different materials have been proposed to support cells and promote their differentiation and proliferation towards the formation of a new tissue or organ. It was demonstrated that polymeric membranes are attractive for their characteristics of selectivity, stability and biocompatibility in the use of biohybrid systems for cell culture. In particular, semipermeable membranes act as supports for the adhesion of anchorage-dependent cells and allow the specific transport of metabolites and nutrients to cells and the removal of catabolites and specific products. Moreover, in order to activate specific biological responses of the cells adhered on the membranes, several approaches including the development of new membranes, surface modification by grafting of functional groups and biomolecule immobilization have been undertaken to enhance the cytocompatibility of membranes. In this paper, we focus on the development of membrane biohybrid systems to be used in tissue engineering and to study disease, drug injection and molecular therapeutics. We review our strategies performed to generating tissue engineered structures, focusing on liver and neurons.
Abstracts: XXXVI Annual ESAO Congress, 2-5 September 2009, Compiègne - France

VASCULAR IMPLANTS

O72 MECHANICAL CHARACTERIZATION OF VASCULAR PROSTHESSES BEFORE AND AFTER IMPLANTATION.

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Objectives: Clinical reports indicate degradation of vascular prosthesis. This research aims at evaluating the mechanical properties of knitted polyester fiber-type (Dacron, Gelsoft Vascutek) and expanded polytetrafluoroethylene-type (Gore-tex) vascular prostheses before and after implantation, and to compare it with the mechanical behavior of normal arteries.

Materials and Methods: We implanted in carotid position two 6mm Dacron and two Gore-tex in four sheep. Axially- and circumferentially-oriented strips were prepared and tested in a uniaxial tensile test bench, before and after 6 months of implantation. Similar strips of a normal sheep carotid artery (table).

Conclusions: The mechanical properties of Dacron- and Gore-tex-type vascular prostheses are not stable, but change significantly after implantation with a trend in reduction of stiffness, particularly for the circumferential direction.

Linearized stiffness at 50% strain axial (MPa) Linearized stiffness at 50% strain circum (MPa) Rupture strength axial (MPa) Rupture strength circum (MPa)
Dacron-Pre (n=2) 5.4±2.0 23.4±0.4 28.4±6.7 47.0±11.9
Dacron-Post (n=2) 7.4±0.8 7.0±0.4 8.3±1.9 8.6±3.6
Gore-tex-Pre (n=2) 3.2±0.4 13.8±1.0 34.8±17.4 32.8±7.4
Gore-tex-Post (n=1) 2.6 2.5 17.6 17.7
Carotis (n=2) 4.4±1.4 1.6±0.6 5.9±0.1 5.8±0.7
Abstracts: XXXVI Annual ESAO Congress, 2-5 September 2009, Compiègne - France

O75

Electrospun small-diameter polyurethane conduits and host cell population

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Objectives: Current approaches to small diameter vascular substitutes are insufficient to ensure long-term graft patency. Electrospinning of polymers offers an interesting technique to fabricate conduits which mimic the structure of native vessels. The objective of this study was to evaluate the biological performance of electrospun small diameter conduits in a rat model.

Materials and Methods: Vascular prostheses composed of polyetherurethane were fabricated by electrospinning. Prostheses were implanted into the abdominal aorta of 40 rats. At 7 days, 4 weeks, 3 or 6 months after surgery specimens were retrieved and evaluated by conventional histology, immunohistochemistry and scanning electron microscopy.

Results: The patency rate of all grafts was 95%. Neither foreign body-type reactions nor anomalies of vessel occlusion were observed. Within 1 week of implantation the midgraft regions revealed immigration of CD 34+ fibroblasts and macrophages. Within 1 month myofibroblasts populated the graft wall. At 3 months after implantation smooth muscle cells and collagen type I positive fibres were detectable in the prosthesis.

Conclusions: Beside biomechanical benefits, electrospun polyurethane grafts exhibit excellent biocompatibility in vivo. Cell immigration and differentiation seems to be promoted by the nanostuctured artificial matrix.

O76

Endocom: A wireless endoprosthesis dedicated to the follow-up of abdominal aortic aneurysms

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6 INRIA, REO Team, Rocquencourt, France; 7 Materials and Methods: The samples were harvested during routine explantation or substitution by other prostheses or during post-mortem examination. The combined use of different methodologies for explants analysis provided complementary information of both the material reaction to the biological environment and the host response to the implant. The study includes histopathology (calcification, vegetations), chemical analysis, surface studies. The implanted valve pathology assessment (infective, degenerative, calcification), with the estimation of the degree of valve insufficiency/stenosis have been performed. The influence of heart valve prosthesis damage on hemodynamic efficiency and state of patient health condition (pathology) and also the complex analysis of valve damage reasons and their consequences have been studied using different modeling methods: physical and computer simulation.

Results: About 70 valves and vessels prostheses have been stored and set up providing the biggest bank of explanted prostheses in Europe. The test of chosen samples gave interesting answers both to physicians and engineers’ questions. The several kinds of valves prostheses damage has been modeled and tested.

Conclusions: The described results can be used in diagnosing patients’ data analysis in case the reason of progressive pathology changes is well known. The simulation-based test procedures are developed to support the explanted valve prostheses studies.

Cryopreservation

O79

Methylnprednisolone and tacrolimus prevent hypothermia-induced endothelial dysfunction – possible use in transplantation surgery


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Objectives: Hypothermia is used to preserve organs and tissue for transplant. Loss of tissue function and edema are common complications after organ transplantation. The present study was designed to examine the effects of methylprednisolone (MP) and tacrolimus (TAC) on endothelial cell function and morphology after deep hypothermia and rewarming, analogous to clinical settings.

Materials and Methods: Human umbilical vein endothelial cells (HUVEC) were pretreated with MP and/or TAC and incubated either within a hollow fiber based perfusion system (SlideReactor) or in monolayers. They were then exposed to a dynamic cooling and re-warming protocol. Immunocytochemistry, time-lapse video microscopy, cell permeability, cell adherence assays and western blot analysis were performed.

Results: Confluent endothelial cells exposed to hypothermia displayed stabilized cell shapes with intercellular gap formation, increased endothelial cell-layer permeability and loss in adherence. Upon re-warming, however, endothelial cell integrity was restored. Opening and closing of intercellular gaps was dependent on ERK 1/2 activation and connexin 43 (Cx43) expression. The combined treatment with MP and TAC inhibited these hypothermia-induced changes.

Conclusions: These results suggest that MP and TAC inhibit hypothermia-induced endothelial gap formation via ePERK 1/2 inhibition and connexin 43 stabilization. Combined application of the two drugs may therefore be considered as a potential new therapeutic strategy to prevent endothelial dysfunction after hypothermia and re-warming during cardiopulmonary surgery.
**Abstracts: XXXVI Annual ESAO Congress, 2-5 September 2009, Compiègne - France**

**O79**

**μ-CRYOPRESERVATION**

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**Objectives:** Cryopreservation plays an important role in the long-term storage of cells and tissue banking and will gain even greater importance when tissue engineering becomes an everyday reality. A high survival rate of cryopreserved cells is a function of optimal cooling rate, appropriate cryoprotective agent (CPA) and its adjusted concentration. Obstacles for the cryopreservation of 3-dimensional samples are inhomogeneous distribution of temperature, cooling rate, and CPA due to slow heat and mass transfer. Furthermore, the most widely used CPAs, dimethyl sulfoxide (DMSO) and glycerol, are toxic in high concentration and have detrimental effects on the cell biological property. Additional processes are necessary to remove the CPA after thawing, which is time consuming and also costly. Therefore it is a matter of great interest to develop new cryoprotective strategies to reduce CPA-concentration in combination with optimal freezing and thawing rates, especially for rare and expensive cells such as stem cells or transgenic cells. In order to develop optimal preservation strategies, a method is required which allows for the investigation with small amounts of medium, low cell numbers and parallel testing.

**Materials and Methods:** The function of the new freezer is based on the “power down” principle. The device is cooled with liquid nitrogen and heated by heating foils. The freezer consists of a system chamber and a chamber cap "power down" principle. The device is cooled with liquid nitrogen and heated with heating elements. The freeze-concentrated tissue. For this purpose we investigated the amino acid proline as potential CPA, since this compatible solute is a natural protectant in cells that undergo stress.

**Results:** Artificial microvascular endothelial cells (HPEC) are used for freezing experiments. Different concentrations of proline (5mM to 100mM) as CPA are being studied in combination with DMSO (0%, 1%, 2.5%, 7.5%, 10% v/v). Cells are frozen either directly with freezing medium containing proline with a 10 minutes equilibration period (method A) or using the solution for 48 hours in a proline (5mM) containing culture medium (method B). Cells are frozen in standard cryovials with 6.65*106 cells/cryovial.

**Results:** Without proline cell survival rate reaches up to 90% with 10% DMSO. With method A a cell survival rate of 90% was achieved with 20mM proline and 2.5% DMSO. Reduction to 5mM as well as increase to 100mM did not give better results. With method B the cell survival rates could be improved up to 90% even without DMSO.

**Conclusions:** Our results show that proline can be used as additional CPA if a short equilibration time of 10min before freezing is applied. With an extended preincubation time proline can be used instead of DMSO. In further studies, the results will be adopted to the cryopreservation of Tissue Engineered Products.

**The project was supported by the Cluster of Excellence “REBIRTH” (DFG).**

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**O82**

**BOVINE PERICARDIUM FREEZE-DRYING MICROSCOPY IN HEART VALVE MATERIAL DEVELOPMENT**

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**Objectives:** The primary objective of freeze-drying is to preserve biological material without damage. The purpose of drying biological tissue, either homografts or heterografts, is the banking of implants for the use in human and veterinary surgery. Freeze-drying can be successfully applied as a method of bovine pericardium preservation and also as a technological device to prepare new materials obtained by chemical modification of native tissues. This paper describes some critical steps in developing heart valve material using freeze-drying microscopy. Process parameters and product characteristics were examined. Mathematical modelling applied to freeze-drying data has been used to simulate the process in order to predict product behavior.

**Materials and Methods:** Freeze-drying microscope. The secondary structure of native and freeze-dried bovine pericardium tissues were determined by Raman spectroscopy. Differential Scanning Calorimetry (DSC) was used to determine the Tg and specific heat (ACp) values of freeze-dried material.

**Results:** Without proline cell survival rate reaches up to 90% with 10% DMSO. Cells are frozen either directly with freezing medium containing proline with a 10 minutes equilibration period (method A) or using the solution for 48 hours in a proline (5mM) containing culture medium (method B). Cells are frozen in standard cryovials with 6.65*106 cells/cryovial.

**Results:** Our results show that proline can be used as additional CPA if a short equilibration time of 10min before freezing is applied. With an extended preincubation time proline can be used instead of DMSO. In further studies, the results will be adopted to the cryopreservation of Tissue Engineered Products. The project was supported by the Cluster of Excellence “REBIRTH” (DFG).

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**O81**

**PROLINE IMPROVES THE CRYOPRESERVATION OF HUMAN ENDOTHelial CELLS FOR TE**

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**Objectives:** Cryopreservation plays an important role in the long-term storage of cells and tissues. A high survival rate of cryopreserved cells requires an optimal cooling rate and the presence of a cryoprotective agent (CPA) in a sufficiently high concentration. The most widely used CPAs, dimethyl sulfoxide (DMSO) and glycerol, however, are toxic at high concentrations and have detrimental effects on the cell biological functioning. Additional process steps are necessary to remove the CPA after thawing, which is time consuming and also costly. Therefore, it is of great interest to develop new cryoprotective strategies to replace the currently used CPAs or to reduce their concentration.

**For this purpose we investigated the amino acid proline as potential CPA, since this compatible solute is a natural protectant in cells that undergo stress.**

**Materials and Methods:** Human pulmonary microvascular endothelial cells (HPMEC) are used for freezing experiments. Different concentrations of proline (5mM to 100mM) as CPA are being studied in combination with DMSO (0%, 1%, 2.5%, 7.5%, 10% v/v). Cells are frozen either directly with freezing medium containing proline with a 10 minutes equilibration period (method A) or using the solution for 48 hours in a proline (5mM) containing culture medium (method B). Cells are frozen in standard cryovials with 6.65*106 cells/cryovial.

**Results:** Without proline cell survival rate reaches up to 90% with 10% DMSO. With method A a cell survival rate of 90% was achieved with 20mM proline and 2.5% DMSO. Reduction to 5mM as well as increase to 100mM did not give better results. With method B the cell survival rates could be improved up to 90% even without DMSO.

**Conclusions:** Our results show that proline can be used as additional CPA if a short equilibration time of 10min before freezing is applied. With an extended preincubation time proline can be used instead of DMSO. In further studies, the results will be adopted to the cryopreservation of Tissue Engineered Products. The project was supported by the Cluster of Excellence “REBIRTH” (DFG).
Abstracts: XXXVI Annual ESAO Congress, 2-5 September 2009, Compiègne - France

O83

CHOLESTEROL REDUCES SUPERCOOLING AND IMPROVES POST-WARMING SURVIVAL OF 3D LIVER CELL SPHEROIDS FOR A BIOARTIFICIAL LIVER

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Objectives: Acute liver failure has high mortality due to donor liver shortages. A bioartificial liver could bridge the gap to transplant or buy time for liver recovery. To be clinically applicable, a long-term storage method is required. Reducing the level of supercooling will improve recovery of cryopreserved liver spheroids (ELS).

Materials and Methods: HepG2s encapsulated in 1% alginate and cultured for 7 days form ELS. ELS were cooled using a controlled rate freezer in 12% DMSO in Celsior ± cholesterol, temperature profile recorded and stored in the vapour phase of liquid nitrogen. ELS were warmed rapidly, DMSO removed and ELS cultured. Viability was assessed with fluorescein diacetate and propidium iodide staining, quantified with imaging software. Cell numbers were quantified by nuclei count. Albumin synthesis, a measure of liver-specific functions, was quantified by ELISA. ELS were assessed at 24, 48 and 72h post-warming; results are expressed as fold change (%) cf. unfrozen ELS. Statistical analyses were performed using SPSS 16.

Results: Supercooling was reduced by 11°C with cholesterol (n=2). Viability was significantly improved at all time points (p<0.01) with cholesterol, except 72h when no difference was apparent as both groups had recovered. Minimum viability was at 24h for both groups (84% with cholesterol, 45% without). Viable cell numbers were also significantly improved (p<0.01) when cholesterol was included. Proliferation rate was maintained cf. ELS over 72h. Without cholesterol, viable cell numbers were 25%, 34% and 41% at 24, 48 and 72h respectively. Albumin production [µg/10⁶ cells/24h] was slightly reduced in both cryopreserved groups cf. unfrozen ELS to ~89% (n=4 separate experiments).

Conclusions: Cholesterol is an effective heterogeneous ice nucleator that reduces supercooling, minimizing intracellular ice formation. By doing so, the yield of viable, functional cells is improved. Further optimisation and upscaling will enable the biomass for a BAL to be available for patients as required.

REGENERATIVE MEDICINE

O84

DEVELOPMENT OF CARDIAC GENE THERAPY FOR refractory HEART FAILURE

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Objectives: Effective cardiac gene therapy for refractory heart failure requires adopting genes that can certainly improve cardiac function and delivering them into the myocardium safely and effectively. We are planning to use genes of calcium cycling proteins and developing effective non-viral vectors that consists of cationic polymer. In this study, we evaluated the transfection efficiency of the vector to the heart.

Materials and Methods: The vector (Blocked Star Vector) consists of nanostructured cationic polyplexes and developing effective non-viral vectors that consists of cationic polymer. In this study, we evaluated in vivo gene transfection efficiency of the vector to the heart.

Results: At 24h after gene transfer, the higher level of LacZ expression was observed in the myocardium injected with the Blocked Star Vector. The mean protein level of SERCA2a in the hearts injected with the Blocked Star Vector was significantly higher than that in normal hearts.

Conclusions: The Blocked Star Vector showed good in vivo gene transfection efficiency to the heart and could be a promising agent for cardiac gene therapy in the near future.

O85

IS TRANEXAMIC ACID AN ALTERNATIVE TO aprotinin IN FIBRIN-BASED TISSUE ENGINEERING?

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Objectives: Fibrin has been studied as a biological scaffold for various tissue engineering applications. One of the most commonly used and most thoroughly evaluated anti-fibrinolytic agents is aprotinin, an active-site serine protease inhibitor. Recent clinical trials have led to the suspension of the worldwide marketing of aprotinin. With regards to the future application of fibrin in tissue engineering, a suitable alternative fibrinolysis-inhibiting drug is necessary. The aim of the present study was to evaluate tranexamic acid (t-AMCA) as an alternative fibrinogen for the development of fibrin-based tissue-engineered structures.

Materials and Methods: The effects of t-AMCA on clot fibrinolysis were spectrophotometrically quantified in vitro. Cytotoxicity, proliferation and apoptosis of carotid artery-derived cells treated with t-AMCA were quantified by using cytotoxicity assay, MIT assay and Caspase-Glo 3/7 assay, respectively. The influence of t-AMCA on the mechanical strength of fibrin gels was measured, and tissue development was analyzed by light and transmission electron microscopy.

Results: The inhibition of fibrinolysis by t-AMCA was comparable to that of aprotinin. In vitro assays showed that t-AMCA (range: 30-160 µg/mL) elicited cytotoxic effect on cultured cells, and did not affect cell proliferation or induce apoptosis. t-AMCA was also shown to have no negative influence on the mechanical stability of fibrin gels. Light and transmission electron microscopy of fibrin gels cultured in medium supplemented with t-AMCA demonstrated good tissue development.

Conclusions: The results of the present study suggest that t-AMCA may be a suitable alternative to aprotinin for controlling the degradation of fibrin-based tissue-engineered cardiovascular structures.

O86

PRO-ADHESIVE SYNTHETIC PEPTIDES ENHANCE IN VITRO CELL ADHESION, PROLIFERATION AND MIGRATION

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Objectives: Despite important advances, the regenerative medicine of the heart valves is still far from creating a lifelong bio-artificial prosthesis. The use of chemically synthesized pro-adhesive peptides sequences is an appealing strategy for the creation of a biomimetic material retaining the biological activities.

Materials and Methods: The bovine pericardium is decellularized with the UTRIDOC method. This acronym refers to a protocol where Urea, Triton X-100 and Sodium Cholate are used in a mild denaturing condition preserving the matrix architecture although a quite strong extraction specifically for the glycosaminoglycans. The bovine pericardium is then irradiated in order to induce the covalent crosslinking of the peptide to the tissue. It is a RGD- peptide 2NSBDG [sequence X[GRGDS]4X; X= Phe(p-N3)], synthesized by a solid phase technique. In addition for quantitative analyses, a fluorescent marker analogue of the peptide was synthesized.

Results: The first result is the stability of the covalently linked peptide inside the tissue once decellularized. The peptide is anchored to some matrix elements that are not affected by the detergent treatment. Similarly in the irradiated and subsequently decellularized pericardium the uronate quantity is higher compared to that belonging to the exclusively decellularized samples. The GAGs, thus, are partially preserved; their presence and the peptide action speed up the repopulation process of the peptide treated samples compared to the just decellularized ones.
Abstracts: XXXVI Annual EASO Congress, 2-5 September 2009, Compiègne - France

Conclusions: This research is still in progress but preliminary data point out that the use of the RGD sequence enhances the repopulation features of the acellular pericardium to be used as starting material for the construction of a new heart valves generation for the clinical practice.

**087**

NEW SYNTHETIC MOLECULES DRIVE CARDIAC AND ENDOTHELIAL FATE IN HUMAN MESENCHYMAL STEM CELLS AND ENHANCE CARDIAC REPAIR IN INFARCTED RAT HEARTS

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**Objectives:** Cardiomyocyte hibernation due to infarct, may cause the progression toward heart failure. Long-term therapeutic solution is heart transplantation, but it can not be the solution for every patient. Developing synthetic molecules that coaxe cell differentiation into desired lineages and establish a complete medium of cardiac repair with days 1, 3, 5, and 7 for histological analyses.

**Results:** HBR dramatically increased the transcription of both cardiac and endothelial genes, and led to the formation of cells expressing vWF or cardiac markers. FMhMSCs transplanted in vivo into infarcted rat hearts differentiated into cardiomyocyte-like elements and endothelial cells, and led to a normalization of left ventricular function. Both tissue rescue and reduction in scar formation were significantly more accentuated in animals receiving FMhMSCs pretreated with HBR.

**Conclusions:** Our findings demonstrate the potential for chemically manipulating a gene program of cardiogenesis in hMSCs and pave the way to new approaches to myocardioc regeneration.

**088**

IN VITRO EVALUATION OF CHEMOKINE RELEASE FROM ELECTROSPUN SCAFFOLDS

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**Objectives:** Pro-angiogenic chemokines, stromal cell-derived factor 1 (SDF-1), and interleukin-8 (IL-8), have been shown to increase the recruitment of many pro-angiogenic cells, such as macrophages. The objective of this study was to quantify the release of SDF-1 and IL-8 from electrospun materials and demonstrate that they have the ability to enhance macrophage recruitment to promote in situ regeneration of bioresorbable vascular grafts.

**Materials and Methods:** Polycaprolactone (PCL, 80 and 150 mg/mL) and silk (70 and 150 mg/mL) were electrospun with and without SDF-1 or IL-8 (3000 ng/mL). 6 mm discs were disinfected, placed in a 96 well plate, and incubated (37°C) with functional SDF-1 and IL-8. ELISA analysis was performed to analyze the chemotactic ability of macrophages from SDF-1 and IL-8 released on silk scaffolds.

**Results:** Silk scaffolds with smaller fiber diameter release undetectable amounts. Release of IL-8 from electrospun scaffolds is significantly higher than on the wall of the right ventricle. Up to now, there is no evaluating system enabling us to measure precisely the mechanical performance without the need for animal experimentation.

**Conclusions:** A realistic heart model may be constructed by scanning a child’s heart with a 3D scanner (NextEngine). The scan data may then be fed into 3D printer (Fab@Home) in order to reproduce an identical 3D model in silicone. The heart model obtained may be fitted with the smart biVAD. Two graduated polyurethane pipettes of different volumes were attached to the main pulmonary and left ventricles before the aortotomy performed and the postoperative release of IL-8 from scaffolds is significantly higher than on the wall of the left ventricle.

**Conclusions:** Artificial muscles for heart compression (AMHC) and sarcomeric actin (α-sarcomeric actin) and vWF proteins were assessed by immunofluorescence. Myocardial infarction was produced in rats by occlusion of the left anterior descending coronary artery. At fourth week cardiac parameters were measured and rats were sacrificed for histochemical analyses.

**Results:** HBR dramatically increased the transcription of both cardiac and endothelial genes, and led to the formation of cells expressing vWF or cardiac markers. FMhMSCs transplanted in vivo into infarcted rat hearts differentiated into cardiomyocyte-like elements and endothelial cells, and led to a normalization of left ventricular function. Both tissue rescue and reduction in scar formation were significantly more accentuated in animals receiving FMhMSCs pretreated with HBR.

**Conclusions:** Our findings demonstrate the potential for chemically manipulating a gene program of cardiogenesis in hMSCs and pave the way to new approaches to myocardioc regeneration.

**089**

EXPERIMENTAL TEST BENCH FOR PEDIATRIC BIVENTRICULAR ASSIST DEVICE BASED ON ARTIFICIAL MUSCLES FOR HEART COMPRESSION

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**Objectives:** We developed a Test Bench to assess the performances of biventricular assist device made of smart materials (smart biVAD). In order to reproduce the physiological functionality of a human heart, the smart biVAD is capable of exerting a much stronger pressure on the wall of the left ventricle to simulate the principle of counterpulsation. Developed in silicone, the heart model obtained may be fitted with the smart biVAD. Two graduated polyurethane pipettes of different volumes were attached to the main pulmonary and left ventricles before the aortotomy performed and the postoperative release of IL-8 from scaffolds is significantly higher than on the wall of the left ventricle.

**Conclusions:** Artificial muscles for heart compression (AMHC) and sarcomeric actin (α-sarcomeric actin) and vWF proteins were assessed by immunofluorescence. Myocardial infarction was produced in rats by occlusion of the left anterior descending coronary artery. At fourth week cardiac parameters were measured and rats were sacrificed for histochemical analyses.

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**Conclusions:** Our findings demonstrate the potential for chemically manipulating a gene program of cardiogenesis in hMSCs and pave the way to new approaches to myocardioc regeneration.

**090**

EFFECT OF ANGLE ON IAB HEMODYNAMICS IN A MOCK CIRCULATION

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**Objectives:** Patients assisted with Intra Aortic Balloon Pump (IABP) are usually nursed in the semi-recumbent position (30-45°) resulting in non-uniform hydrostatic pressure distribution along the IAB. The aim of this study is to evaluate hemodynamics of IAB in a mock circulation (MC) resembling the effect of patient’s posture.

**Materials and Methods:** A realistic heart model may be constructed by scanning a child’s heart with a 3D scanner (NextEngine). The scan data may then be fed into 3D printer (Fab@Home) in order to reproduce an identical 3D model in silicone. The heart model obtained may be fitted with the smart biVAD. Two graduated polyurethane pipettes of different volumes were attached to the main pulmonary and left ventricles before the aortotomy performed and the postoperative release of IL-8 from scaffolds is significantly higher than on the wall of the left ventricle.

**Results:** The silicon heart model has an end-diastolic volume of 50cc on the left side and 52cc on the right side. The after load ranges from 20 to 100mmHg on each sides and independently. The ultrasonic probe has an operational range of 25cm. The maximal volume ejected per systole detectable is 40cc corresponding to an ejection fraction of 80%. For this, developed a Test Bench to assess the performances of biventricular assist device made of smart materials (smart biVAD). In order to reproduce the physiological functionality of a human heart, the smart biVAD is capable of exerting a much stronger pressure on the wall of the left ventricle to simulate the principle of counterpulsation. Developed in silicone, the heart model obtained may be fitted with the smart biVAD. Two graduated polyurethane pipettes of different volumes were attached to the main pulmonary and left ventricles before the aortotomy performed and the postoperative release of IL-8 from scaffolds is significantly higher than on the wall of the left ventricle.

**Conclusions:** Artificial muscles for heart compression (AMHC) and sarcomeric actin (α-sarcomeric actin) and vWF proteins were assessed by immunofluorescence. Myocardial infarction was produced in rats by occlusion of the left anterior descending coronary artery. At fourth week cardiac parameters were measured and rats were sacrificed for histochemical analyses.

**Results:** HBR dramatically increased the transcription of both cardiac and endothelial genes, and led to the formation of cells expressing vWF or cardiac markers. FMhMSCs transplanted in vivo into infarcted rat hearts differentiated into cardiomyocyte-like elements and endothelial cells, and led to a normalization of left ventricular function. Both tissue rescue and reduction in scar formation were significantly more accentuated in animals receiving FMhMSCs pretreated with HBR.

**Conclusions:** Our findings demonstrate the potential for chemically manipulating a gene program of cardiogenesis in hMSCs and pave the way to new approaches to myocardioc regeneration.

**091**

DEVICE BASED ON ARTIFICIAL MUSCLES FOR HEART COMPRESSION

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**Objectives:** We developed a Test Bench to assess the performances of biventricular assist device made of smart materials (smart biVAD). In order to reproduce the physiological functionality of a human heart, the smart biVAD is capable of exerting a much stronger pressure on the wall of the left ventricle to simulate the principle of counterpulsation. Developed in silicone, the heart model obtained may be fitted with the smart biVAD. Two graduated polyurethane pipettes of different volumes were attached to the main pulmonary and left ventricles before the aortotomy performed and the postoperative release of IL-8 from scaffolds is significantly higher than on the wall of the left ventricle.

**Conclusions:** Artificial muscles for heart compression (AMHC) and sarcomeric actin (α-sarcomeric actin) and vWF proteins were assessed by immunofluorescence. Myocardial infarction was produced in rats by occlusion of the left anterior descending coronary artery. At fourth week cardiac parameters were measured and rats were sacrificed for histochemical analyses.

**Results:** HBR dramatically increased the transcription of both cardiac and endothelial genes, and led to the formation of cells expressing vWF or cardiac markers. FMhMSCs transplanted in vivo into infarcted rat hearts differentiated into cardiomyocyte-like elements and endothelial cells, and led to a normalization of left ventricular function. Both tissue rescue and reduction in scar formation were significantly more accentuated in animals receiving FMhMSCs pretreated with HBR.

**Conclusions:** Our findings demonstrate the potential for chemically manipulating a gene program of cardiogenesis in hMSCs and pave the way to new approaches to myocardioc regeneration.
substantial at 1.1 [20.3±0.72 vs 9.6±0.95 (1:2) and 10±0.4 (1:3) mmHg] but is lost with increasing angle from 0° to 45° [20.3±0.72 vs -8±0.67 mmHg]. The WIA compression and expansion waves associated respectively with inflation and deflation carry less energy at higher angles, reducing from 0 to 45° [0.121±0.0027 vs 0.055±0.0008 (compression) and 0.202±0.005 vs 0.054±0.0036 (expansion) J/m²]. The compression wave correlates significantly with diastolic P augmentation (R=0.78).

Conclusions: Assisting frequency of 1:1 shows better hemodynamics in terms of P but not Q. IAB hemodynamics are affected by posture, especially in terms of diastolic augmentation and end-diastolic reduction. This suggests that higher operating angles may reduce the benefit of counterpulsation.

O91
REALISING AN INTRA-AORTIC VAD CONCEPT
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Objectives: The increasing global prevalence of congestive heart failure and advent of various technologies to allowed ventricular assist devices (VAD) to become more reliable and versatile in recent times. As an example of realizing the next generation of VADs for targeted therapy, we report on the development of an intra-aortic axial pump to be placed in the descending aorta. The device purpose is to augment blood flow to the abdominal organs to sustain life.

Materials and Methods: A prototype device was designed and manufactured using a combination of engineering principles in motor design, fluid dynamics and physiology. The proposed VAD required operation in synchrony with the natural heart due to the proximal placement in the descending aorta. An extended computational model of the human circulatory system (HCS) was initially used to explore the interaction between the HCS and VAD, and ultimately to define parameters for a prototype. The design of the axial impeller for the specific application was optimized by turbomachine theory. To overcome the challenge of a large gap between the impeller/rotor and stator, an ultra-light ironless motor was used to drive the pump. The prototype was then tested in a hybrid mock circulatory system for both local hemodynamic and global circulatory system studies. The mock loop, having both physical and numerically simulated components, provides detailed HCS response profiles to the pump axial flow, enabling the refinement of the pump.

Results: Various pump characteristics including impeller speed profiles, regional circulatory pressure and flow responses, and hydraulic performance evaluations (stall pressure of 20.9 mmHg at 7.518 rpm) are presented.

Conclusions: The design of a minimally-invasive axial VAD concept was evaluated, and mock loop experiments demonstrated device synchronization with the natural heartbeat while boosting blood flow. Future works include further in vitro studies including hemolysis experiments.

O92
INVESTIGATION OF THE FRANK-STARRLING-LIKE BEHAVIOUR OF THE BIVACOR ROTARY BIVAD/TAH
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Objectives: Ventricular assist devices are generally poorly equipped to treat patients with end-stage biventricular failure. Large, cumbersome, and often extracorporeal BIVAD therapy and/or pulsatile total artificial hearts are required. This study presents the in vitro and in vivo results of a 3rd generation rotary BIVAD/TAH. The BIVACORTM is a wear-free, single unit centrifugal pump, measuring just 70mm x 60mm. It is capable of providing full Frank-Starling-like support to the systemic and pulmonary circulatory systems by means of a unique, axially displaceable, double impeller design.

Materials and Methods: A series of 9 acute in vivo (sheep) trials were conducted to test the functional response of the “dual pumping” device to a number of clinical conditions during biventricular assistance and total heart replacement. The unique ability of the device to mimic the Frank-Starling effect and prevent ventricular collapse, by automatically altering the left/right chamber with impeller axial displacements in response to changing venous return, was also evaluated. In vitro tests confirmed the devices sensitivity to ventricular preload changes, and its ability to correspondingly alter device outflow.

Results: Simultaneous alteration of the left/right outflow was successful in matching the flow requirements of both circulatory systems and maintaining adequate atrial filling pressures in the acute animal study. Changes in ventricular preload were met with an automatic alteration of impeller position, which produced a corresponding change of outflow.

Conclusions: The systemic and pulmonary circulation of each animal was successfully supported during conditions of normal, failing and absent heart function. The automatic Frank-Starling-like feature of the device was quantified in vitro. This device will provide a suitably miniature and relatively inexpensive long-term rotary device for patients who require bi-ventricular support and/or total heart replacement.

O93
DEVELOPMENT OF A NOVEL RESPIRATORY ASSIST CATHETER
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Objectives: Respiratory assist using an intravascular catheter employing a hollow fiber bundle may be a potential treatment for patients suffering from acute or acute-on-chronic lung failure. A potential barrier has been developing a small enough catheter for simple percutaneous insertion. The objective of this study was to develop and test a novel respiratory assist catheter that uses HEPA within the fiber bundle to actively mix blood and enhance gas exchange efficiency, thus requiring a smaller fiber bundle and insertional size for the catheter.

Materials and Methods: Respiratory catheters were fabricated with a 25 French insertional size based on nine different impeller designs. Bench testing of gas exchange in deionized water in a mock veno cava test section was used to indentify the three best performing impeller designs for gas exchange efficiency. Respiratory catheters with the three best performing impeller designs were evaluated in acute studies in four calves (122 ± 10 kg).

Results: Gas exchange increased significantly with increasing impeller rotation rate. The degree of enhancement varied with impeller geometry. The maximum gas exchange efficiency (exchange per unit surface area) for the catheter with the best performing impeller was 529 ± 20 ml CO2/min/m² and 513 ± 21 mL CO2/min/m² for bench and animal studies, respectively, at a rotation rate of 20,000 RPM. Given the bundle size of catheters, absolute CO2 exchange was 37 and 36 mL CO2/min respectively.

Conclusions: There was a strong correlation between gas exchange in the bench studies and in the acute animal implants. Active mixing by rotating impellers produced 70% higher gas exchange efficiency than past designs based on pulsating balloons within the catheter fiber bundle. The sensitivity of gas exchange to impeller design suggests that further improvements can be made by CFD-based optimization of the impeller, which is currently underway.

BIOARTIFICIAL ORGANS

O94
NEW SUPPORTIVE DEVICE TO RESTORE THE LUNG ELASTIC RECOIL IN COPD
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Objectives: Chronic obstructive pulmonary disease (COPD) is a progressive disorder with poor prognosis and expected to be the third leading cause of death by 2020. There is no effective treatment to improve the prognosis of the patients at present. We propose a novel approach for this disease. COPD is characterized by the airway flow limitation and loss of elastic recoil of the lung. We designed elastic net, which covers the lung to restore the elastic recoil. Here we report its effect on the lung mechanical properties.

Methods: The net is composed of nylon and polyurethane, whose spring rate is 2.5Kgf/m. Based on the 3D CT scan data, the elastic net was given a shape that fits a canine lung. The mechanical properties of the three canine unilateral lung were measured before and after the net covering.

Results: The static and the dynamic pressure-volume curves were determined with the data obtained from the EVITA XL™ mechanical ventilator. A rightward shift of the pressure volume curve was observed after the net covering. The average static lung elasticity was increased by 26% and the dynamic lung elasticity was also increased by 24% after the net covering. The elastic energy stored during the inspiration showed 20% of augmentation.
Conclusions: The elastic net proved the lung elasticity increase and potentially improve the respiratory failure in COPD patients.

**095 ANTIBACTERIAL EFFECTS OF BIOACTIVE GLASS NANOPOWDERS WITH DIFFERENT COMPOSITIONS**
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Objectives: Bioactive glass has potential as a bone replacement graft biomaterial and is effective for the treatment of bone defects. The antibacterial action of a bioactive glass is affected by its chemical composition. Nanoscale biomaterials offer improved performances due to their large surface to volume ratio and unusual chemical behavior. The aim of this work was to prepare, characterize and evaluate the antibacterial effects of bioactive glass nanopowders.

Materials and Methods: Bioactive glasses with three different compositions (58S, 65S, 72S) were prepared via sol-gel technique. Characterization techniques such as X-ray diffraction (XRD) and Transmission electron microscopy were utilized in order to phase analysis and study of the structure and particle size of synthesized bioactive glasses. Chemical compositions of the obtained nanopowders were determined by X-ray fluorescence. The antibacterial activity of the obtained bioactive glass nanopowders were studied using Escherichia coli, Pseudomonas aeruginos, Salmonella typhi, and Staphylococcus aureus.

Results: The compositions of prepared bioactive glasses were similar to the predicted compositions and the obtained bioactive glasses had the particle sizes less than 100 nanometers. The 58S bioactive glass showed the highest antibacterial activity and its minimum bactericidal concentrations (MBC) for E. coli, S. aureus, and P. aeruginosa were 50 and 100 mg/mL, respectively. 65S exhibited antibacterial and bacteriostatic effects on E. coli, and S. aureus at concentrations of 100 and 50 mg/mL, respectively, while its MBC was 100 mg/mL. However, 72S bioactive glass showed no antibacterial effect.

Conclusions: Antibacterial activity of bioactive glass could be improved by decreasing the particle size and optimizing the chemical composition. Bioactive glass with bioactivity and antibacterial characteristics could be considered as a good candidate for treatment of oral bone defects, bone replacement, and tissue scaffold applications.

**096 LASER-INDUCED CELL SEEDING OF COLLAGEN SCAFFOLDS**
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Objectives: Cell seeding is the first step to establish a high quality tissue-engineered product (TEP). A novel laser approach was used for the three-dimensional cell seeding of collagen scaffolds. With the laser-induced forward transfer (LIFT) cells can be positioned in a defined manner in 3D-constructs by using a laser pulse. This direct-writing technique allows defined cell transfer in various patterns.

Materials and Methods: NIH-3T3 cells were cultivated in RPMI-Medium with 10% (v/v) FCS and 1% (v/v) Pen./Strep. Biodegradable collagen scaffolds produced by directional solidification and subsequent freeze-drying with a homogeneous pore sizes of 100µm were used as carrying structure for the NIH-3T3 cells. 50µl of the cell suspension with various cell concentrations were applied as a thin biolayer on a substrate holder placed parallel and near the scaffold. A laser pulse were transferred the cell into the scaffold. The viability of the laser transferred cells and their distribution in the scaffold were detected by Calcein AM and Ethidium homodimer staining directly after printing and, additionally, after 4 weeks’ cultivation.

Results: After laser-induced forward transfer living cells could be detected within the scaffold by Calcein AM staining. The cell density was found to be greater on the rim as compared to the scaffold centre. The direct-writing technique allows to position at different depth ranges in the scaffold. It was found that the cells were transferred on the collagen matrix showed their typical cell shape. A few hours after cell transfer proliferation could be noticed. A long-term cultivation of the cell seeded scaffolds for 4 weeks showed continuous cell proliferation of the laser transferred cells.

Conclusions: Cell seeding of collagen scaffolds via laser-induced forward transfer is promising and offers new seeding options for 3D-constructs in regenerative medicine.

**097 EVALUATION OF WISTAR RATS MESENCHYMAL STEM CELLS RESPONSE TO NOVEL GELATIN SCAFFOLD COATED WITH NANO ROD HAP**
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Objectives: This study is to examine the response of mesenchymal stem cell wistar rats (WMSCs) on novel gelatin scaffold coated with nano rod HAP (nrHAp). The attachment and the proliferation behavior of the cells on gelatin scaffold coated with nrHAp were assessed in comparison with those on pure gelatin scaffold in growth medium DMEM (Dulbecco’s Modified Eagle Medium) supplemented with 15% FBS (Fetal Bovine Serum), 100U/mL streptomycin/penicillin antibiotics. WMSCs attached and proliferated on the prepared scaffold. The scaffold coated with nrHAp has high potential for use in bone tissue engineering and repair.

Materials and Methods: The materials were gelatin, Gelita; carbodiimide derivative, Merck; DMEM, FBS, streptomycin/penicillin antibiotics, Gibco. Porous gelatin scaffolds mixed with nrHAp particles were obtained by freeze drying and crosslinked by carbodiimide derivative. The scaffolds were coated by pressing into a nrHAp solution. WMSCs were seeded on surface of the scaffolds. The cultures were provided with DMEM medium and incubated in CO2 at 37°C for 7 days.

Results: The SEM micrographs show that WMSCs occupy internal spaces and attach on the scaffold surfaces. In most areas the cells had cuboidal morphology and in some rare regions fibroblastic. Also, there was a cytoplasmic bridge between two adjacent cells. MTT assays were performed on day 4 and 7. Statistical comparison indicated that there was a significant difference between coated and uncoated scaffolds. This could be attributed to the presence of the nrHAp particles on the scaffolds surface.

Conclusions: According to our results, coating of scaffolds with nrHAp particles enables the prepared scaffolds to possess desirable biocompatibility, high bioactivity, better cell attachment and proliferation. This research suggests that the newly developed scaffold has a potential as a suitable scaffold for bone tissue engineering.

**098 CHARACTERIZATION OF SELF-ORGANIZING STRUCTURES IN AN OXYGEN TRANSPORT EMULSION**
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Objectives: To assure effectiveness in blood substitutes (biocompatibility and a good oxygen uptake), the emulsion could not present changes in stability or particle size distribution during lifetime. The objective of this study is to identify the kind of particles present in an oxygen transport emulsion, in order to analyze the interactions between them and their global effect on the stability of the dispersion.

Materials and Methods: The emulsion is composed of Perfluorocarbon, PFC (Perfluorooctyl Bromide 99%, Exfluor Research Corp, USA), Lecithin (Epbikuron 1707°, DEGUSSA, Germany) and an aqueous phase with additives to control viscosity, pH and emulsify. The evolution of particle size is characterized by two different techniques, dynamic light scattering (a direct method) and near infrared spectroscopy (an indirect method).

Results: Immediately after preparation, the emulsion has a multimodal particle size distribution, as a consequence of the presence of more than one kind of particles (unilamelar liposomes and PFC droplets). In the multimodal distribution each population can be associated to a specific kind of particle. This association is established through the comparison between a free PFC dispersion and a PFC emulsion. Also, in PFC-free dispersion it has been observed that some particular populations appear and disappear, indicating the formation of temporal aggregates of liposomes. Moreover, sedimentation in free PFC dispersion is observed after two weeks of preparation, maybe because small liposomes become multimamellar liposomes.

Conclusions: It is possible that the simultaneous presence of unilamelar liposomes, multimamellar liposomes and PFC droplets in PFC emulsions changes the instabilities mechanisms of a typical emulsion.
TISSUE ENGINEERING, BIOREACTORS

O09 AUTOLOGOUS ORAL MUCOSAL EPITHELIAL CELL SHEET FOR CORNEAL EPITHELIAL RECONSTRUCTION
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Objectives: By definition, the use of autologous limbus or limbal epithelium is impossible for the transplant surgery for bilateral limbal epithelial stem cell deficiency (LSCD). Allogenic limbal epithium from living-related donors necessitates postoperative immuno-suppression responsible of secondary adverse effects. Cultured autologous oral mucosal epithelial cells sharing corneal markers with cornea epithelial cells have been proposed as an alternative treatment for LSCD. We present the promising intermediary results of 15 patients grafted with novel tissue-engineered product, CAOMECS suffering from bilateral total limbal stem-cell deficiency with severe loss of vision (<1/10).

Materials and Methods: After cell isolation, epithelial cells are seeded on UpCell® (Inserm CellSeed Inc) and cultured in keratinocytes medium. 4 days after confluence, this intelligent polymer, by reducing the temperature of the culture, allows complete detachment of the cultured cells and enzymatic treatment preserving the basement membrane and cell-cell junction. CAOMECS is harvested as a transparent, undamaged, strong, viable and rapid bio-adhesive cell-sheet allowing the graft on corneal stroma without suturing.

Results: CAOMECS is well tolerated, but one patient with severe Lyell Syndrome showed a predictable inflammatory adverse event classified as “not product related”. As follow-up observation after more than 15 months since the first-patient-graft, 13/15 patients present no more ulcers, no vessels anymore or decrease of neovessels, and decrease of PEK in any cases. The recurrence of neovessels on 4/15 cases after 8 months follow-up can be due to a too long carrying of lens or a severe state of the stroma.

In all cases, patients felt a better comfort (less pain, dryness or photophobia). Concerning visual acuity, at least 1 level increase was shown in more than 50% patients.

Conclusions: CAOMECS is a well tolerated and safe tissue-engineered product for the treatment of human LSCD. Efficacy and persistence of CAOMECS for 17 months to date and follow-up monitoring is continuing. At post-CAOMECS treatment, a donor corneal graft with a high probability of no rejection has been done in the first three blind patients, and has demonstrated an increase in visual acuity on these patients.

O100 CARTILAGE ENGINEERING USING PHARMACOLOGICALLY ACTIVE MICROCARRIERS COMBINED WITH MESENCHYMAL STEM CELLS
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Objectives: Pharmacologically Active Microcarriers (PAM) are poly(D,L lactide-co-lycolide) biocompatible and biodegradable microparticles, that may convey cells on their surface to provide an adequate 3D microenvironment and deliver a growth factor to induce or maintain tissue specific differentiation. Our objective was to implement the PAM for tissue engineering of human mesenchymal stem cells (MSC) to stimulate chondrogenesis.

Methods: We set-up a formulation of PAMs enabling the continuous release of any desired growth factor from the microparticles by developing a simple and reversible method to precipitate proteins and limit protein-polymer interactions for efficient encapsulation. To optimize the release kinetics, a biocompatible additive (poloxamer) was chosen using a protein/polymers adsorption model. The in vitro release of TGF-beta3 from the PAM was measured by ELISA and its activity was tested using a specific bioassay. Chondrogenesis was induced by culture of 1.5x105 MSC with 0.5 mg PAMs in chondrogenic medium on low attachment culture plates for 21 days. Expression of the lineage specific markers was quantified by RT-qPCR and immunohistochemistry using anti-collagen 2 and anti-aggrecan antibodies on paraffin sections. For in vivo experimentation, MSC were incubated for 24h with PAMs and injected (10µL) into the knee joints of SCID mice for 3 weeks.

Results: The most efficient molecules for the biomimetic surface of the PAMs were fibronectin and poly-D-lysine both for cell adhesion and survival of MSC in vitro. The in vitro release of TGF-beta3 reached 22% of the total amount of encapsulated protein by the first week and a plateau was observed after approximately 30% release by the first month. Importantly, more than 85% of released TGF-beta3 was functionally active. When MSC were cultured in presence of PAM-TGF, cells rapidly adhered onto the PAMs and progressively aggregated to form a unique pellet-like structure from day 7 to day 21. In PAM-TGF-induced aggregates, high expression of chondrogenic markers occurred in a time-dependent manner whereas expression of osteogenic and adipogenic markers was lower than those observed when PAM-FN were used. An intra-articular injection of MSC mixed with PAM-TGF confirmed their capacity to form a neotissue with characteristics of cartilaginous-like tissue.

Conclusions: The combination of PAMs with TGF-beta3 allows MSC to preferentially differentiate into chondrocytes. Indeed, PAMs represent a promising strategy for delivering bioactive molecules that may be useful for tissue engineering.

O101 ACCELERATED PRODUCTION OF BIOHYBRID BONE SUBSTITUTES WITHIN BIOREACTOR-BASED SYSTEMS
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Objectives: Bone tissue engineering is a promising domain that may soon provide valuable solutions to patients in need of bone reconstruction. Our project aims at developing bioreactor-based systems dedicated to the production of autologous bone substitutes ex vivo. They may indeed speed up and direct cell proliferation, differentiation and the production of a well-organized tissue. The purpose of this study was to demonstrate the feasibility of culturing bone cells on monolayers of BCP granules within plane-parallel chambers in both static and dynamic conditions, and to characterize the fluidic environment.

Methods: hFOB1.19 or MC3T3 cells were grown for 2 weeks under static conditions. They were cultured for 3 additional weeks while divided into subgroups: one maintained in the static environment, the other included within perfusion loops. Cell counting was performed using Alamar Blue Assays. Cell proliferation was measured using TUNEL assays. Calcium, phosphate and glucose were monitored in the medium throughout the experiment. Results obtained in the dynamic setting were compared to the static controls. CFD modeling was used to investigate fluid distribution and velocity, as well as shear stresses, parameters that may greatly impact cell proliferation and differentiation.

Results: MC3T3 cells attached, proliferated and rapidly formed 3D tissues with mineralization nodules in static. Cell survival and viability were impacted by perfusion. Fluid distribution was shown to be relatively homogeneous, although higher fluid velocities and shear stresses were observed close to the inlet/ outlets channels.

Conclusions: Monolayers of BCP granules provide favorable substrates to bone cells. In the static setting, well-organized 3D constructs were formed after 5 weeks. A patent effect of perfusion was observed on both types of cells.

O102 STRAIN STIMULATION FOR MUSCLE TISSUE ENGINEERING
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Objectives: Our tissue-engineering group focuses on the development of contractile biobconstructs to be used as bandage for akinetic myocardium. In the present study, we aim to define in vitro dynamic culture conditions to induce a cell density, cell organization and mechanical properties of the construct. Accordingly, we report our ongoing study on the development of a new device for the generation of stretch culture conditions.

Materials and Methods: Latex or custom made silicon bulbs (produced with a water-soluble wax mold) were covered with electrospun poly-caprolactone (PCL) nanoscale fiber matrix. Pump controlled volumetric changes induced bulb enlargement and resulted in matrix stretching. Characterization of the
stretch/strain was analyzed using 3D digitizer. C2C12 cells seeding on the 3D matrix was optimized. Cells were cultured for 1 week in basic culture conditions under mechanical stimulation. Static, ramp (25%) and cyclic (1.5 Hz) strains were applied. Cellular responses were investigated by scanning electron microscopy, immunostaining and 3D confocal analysis.

**Results:** Bulb compliance was dependent on the silicone quality and on parallel or serial production. Strain increased from basic to apex of the bulb after 10% changes in volume of the fluid-filled bulb. Rotation during cell seeding resulted in a homogenous distribution of cells that covered the 3D matrix. Preliminary data showed that compared to static culture conditions, dynamic culture induced cellular multilayer formation. In addition, cyclic strains improved cell orientation and ramp strains induced a 2-time increase in tissue thickness from 13 to 28 µm.

**Conclusions:** Dynamic conditioning of nanofibers matrices/myoblast-based tissue engineering using bioreactor and biocompatible matrix was optimized. Cells were cultured for 1 week in basic culture and improved cell orientation and ramp strains induced a 2-time increase in tissue thickness and structure.

**O103 PROLIFERATION AND DIFFERENTIATION OF BONE MARROW DERIVED MENCEHYMAL STROMAL CELLS WITHIN ALGINATE MICROBEADS AND CRYOGEL SPONGES**

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**Objectives:** Alginate gels are currently being employed and explored for a wide range of medical and pharmaceutical applications including tissue engineering. Promising cellular components for tissue engineering are mesenchymal stromal cells (MSC) due to self-renewal and capacity to differentiation into several cell lineages. The aim of the study was the investigation of peculiar properties of human bone marrow MSC growth and differentiation within the alginate microbeads and macroporous cryogel sponges with modified pore surface.

**Materials and Methods:** MSC were isolated from adult human bone marrow, expanded in vitro and then either encapsulated into alginate microbeads or seeded into wide-porous alginate-based sponges. Proliferative activity of MSC within scaffold’s was assessed by Alamar® blue assay. Differentiation of MSC was made by the addition of adipogenic, osteogenic or chondrogenic induction stimulus.

**Results:** After encapsulation of MSC to alginate microbeads or seeding to the wide-porous alginate scaffolds cells maintained spherical shape during culture in expansion medium and did not adhere and proliferate. The modification of the porous scaffold by either the direct incorporation or chemical coupling of gelatin type B to the pore surfaces showed that the direct incorporation of gelatin over the range from 0.125% to 0.5% to the alginate matrix did not provide adhesion and proliferation of MSC within the scaffold. At the same time the covalent attachment of anchor gelatin particles to the pore surface allowed to improve the adherence abilities of the scaffold. During following culture MSC proliferated and distributed within the scaffold. Differentiation studies showed that MSC grown both within the alginate microbeads and wide-porous scaffolds were able to differentiate into adipogenic, osteogenic and chondrogenic cell lineages in response to specific induction stimulus.

**O104 THE STUDY OF ANGIOGENESIS IN FIBRIN-BASED TISSUE ENGINEERED CONSTRUCTS**

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**Objectives:** Tissue engineering as an interdisciplinary field enables the development of autologous tissue by combining cellular and molecular biology as well as material and mechanical engineering in order to replace damaged or diseased organs. The development of complex tissue engineered constructs is limited to a thickness of less than 2mm. To avoid the limitations of diffusion, an internal capillary network is necessary to enable gas exchange, nutrient delivery and waste removal.

The aim of the current study was to develop a bioreactor for the study of angiogenesis within fibrin gel scaffolds seeded with endothelial cells (EC). The flow perfusion bioreactor utilizes the tube forming activity of endothelial cells and the permeability of a fibrin gel scaffold for the formation of capillary networks, supplemented by growth factors.

**Materials and Methods:** The bioreactor is composed of two coaxial cylinders. The fibrin gel scaffold seeded ECs is placed in a scaffold holder and attached directly to the flow system composed of the inner cylinder. This system is housed in a second cylinder connected to an external pump via silicon tubing in order to oxygenate the tissue and eliminate CO2.

**Results:** Preliminary tests show the practicability of the bioreactor as a system for the successful cultivation of cell-seeded gels. The nanostructure of the fibrin gel allows adequate filtration already under low pressure. The actual filtration rate has an average value of 7 mL per hour. The supplement of follicular fluid as VEGF donator induces proliferation and orientation of HUVECs in the fibrin gel.

**Conclusions:** The present study reports the development of a flow bioreactor system for the engineering of capillary networks within a 3D fibrin gel scaffold seeded with endothelial cells. In a preliminary study we could show that follicular fluids induce capillary formation of HUVECs imbedded in a fibrin gel scaffold.

**O105 FIXED BED REACTOR SYSTEMS FOR THE EXPANSION AND DIFFERENTIATION OF STEM CELLS FOR CELL THERAPY**

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**Objectives:** Human mesenchymal stem cells (hMSC) are qualified for cell therapy. hMSC-TERT are modified by transfection with a telomerase activity. The alginate-encapsulated stem cell line is an implantable therapeutic cell system which possesses the potential to counteract endocrine deficiencies in vivo. In the special case of cell therapy the cells have to be differentiated prior to use. An automated GMP conformance process includes three steps: (1) the expansion of the hMSC-TERT (2) the encapsulation of the harvested cells and (3) the differentiation of the encapsulated cells. For the first step the suitability of various nonporous microcarriers for cultivation of hMSC with a harvesting procedure using trypsin, accutase and collagenase was investigated. The yield and the vitality of the cells after harvesting are of particular interest.

**Materials and Methods:** For the cultivation of the human mesenchymal stem cells (hMSC) we used fixed bed reactor systems at different scales. The cultivation of the encapsulated cells was carried out in fixed bed bioreactors based on commercial syringes.

**Results and Conclusions:** The expansion of human mesenchymal stem cells on nonporous microcarriers is preferable when the cells need to be kept in viable condition. The qualification of disposable plastic syringes as small scale single-use fixed bed reactors for the cultivation and differentiation of the encapsulated cells showed, compared to the reference cultures, no disadvantage concerning the viability and differentiation potential. The use of fixed bed reactor systems was successfully introduced as disposable small-scale fixed bed bioreactors for the cultivation and differentiation of implantable therapeutic cell systems.

**O106 DEVELOPMENT OF AUTOLOGOUS TISSUE VASCULAR GRAFTS “BIOTUBES”**

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**Objectives:** We have described that in vivo tissue-engineered autologous tubular tissues “BIOTUBES” could be ideal small-caliber vascular grafts in the animal experiments (Watanabe et al., ESAO2005~2008). They are constructed from recipient bodies safely and economically without any use of special clean facilities. In this study, we summarize the development of BIOTUBES.

**Methods:** Silicone molds (diameter: 2.5~5 mm, length: 20~50 mm) were covered with “sutting-reinforcement” PU sponge cuffs. The assembled scaffolds were implanted into dorsal subcutaneous pouches of Japanese white rabbits or Beagle dogs. After 1 month, BIOTUBES formed around the molds were auto-implanted to the carotid arteries of the same animals.
Results: After 1-month preparation in the subcutaneous tissues of both species, the molds were completely covered with autologous connective tissues (thickness: ca. 0.1mm) mainly consisting of fibroblasts and collagen fibers. The PU sponge cushions enabled easy end-to-end Anastomosis. Japan white rabbit; During 26 week-implantation, neither formation of aneurysms nor rupturing was observed in 2mm diameter BIOTUBEs. Little thrombus was formed on the luminal surfaces completely covered with endothelial cells with parallel orientation to the direction of blood flow at 2 weeks. With time, hierarchical arterial structures were reconstructed in the recipient body under hemodynamic conditions, including circumferentially oriented smooth muscle cells and collagen fibers bundles, and elastin formation. Beagle dog: Angiography up to 4-week implantation showed 100% patency. Longest follow-up in 2 years without any degenerative changes.

Conclusions: BIOTUBEs exhibited excellent performances as small caliber vascular prostheses. They could be applicable as large animals with high-pressure circulation systems including humans. We are now developing bifurcated BIOTUBEs for Y-graft replacement of the abdominal aorta of beagle dogs.

APERESIS AND INSTRUMENTATION

O107 IMMOBILIZATION OF HEPARIN ON POLYSULFONE MEMBRANES FOR PREFERENTIAL ADSORPTION OF LOW-DENSITY LIPOPROTEIN (LDL) XI.J. Cao1, R. Li2, D. Guduru2, T. Groth2, J. Vienken3, T. Beier2
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Objectives: The abnormal elevated level of low-density lipoprotein (LDL) in human blood has been confirmed to be a main risk factor for the process of atherosclerosis. The reduction of LDL level in blood is used therapeutically to lower the risk of cardiovascular-cerebrovascular diseases for example by complexation of LDL with heparin during HELP apheresis. In this study a covalent modification of polysulfone (PSu) membrane with heparin is suggested to achieve a preferential adsorption of LDL from human plasma.

Materials and Methods: Pure polysulfone was supplied by Fresenius Medical Care and used for preparation of flat membranes by phase inversion. PSu membranes were activated by immersion in a solution of chlorodimethyl ether, hexane and SnCl4, at 25°C and then washed with methanol. The chloromethylated PSu membrane was subsequently incubated into ethylenediamine at 25°C to obtain amino groups for the subsequent chemical binding of heparin, which was achieved by biofunctional linker molecules. The heparin density on PSu membrane surface was determined using the toluidine blue method. The hydrophilicity of membranes was characterized on the basis of water contact angle (WCA) measurement. LDL adsorption on PSu film was investigated by a modified enzyme immunoassay (EIA).

Results: The primary activation of PSu membranes by chlorine groups was the main factor to affect the immobilization degree of heparin. A heparin density up to 0.5 µg/cm² on the dense PSu film and 2.0 µg/cm² on the PSu membrane could be achieved. The hydrophilicity of the PSu membrane was improved greatly by covalent immobilization of heparin. The WCA of PSu films was decreased from 86.8° ± 3.7 to 50.5° ± 3.2 after binding of heparin with a quantity of 0.36 µg/cm². The EIA assay was carried out after immersion of membranes in LDL containing solutions. It was observed that LDL adsorbed also to some extent on the hydrophobic PSu surfaces. However, after heparin immobilization when the membrane became quite hydrophilic, significantly higher quantities of LDL were adsorbed.

Conclusions: Heparin could be covalently immobilized onto the PSu membrane by a three-step synthesis method. The modified PSu membrane with heparin acquired an improved ability to adsorb LDL preferentially.

O108 PROOF OF CONCEPT TO ENRICH CIRCULATING TUMOR CELLS USING LEUKAPHERESIS AND ELUTRIATION R.L. Effler1,2, J. Lind1, M. B. Fischer1, V. Weber1, D. Falkenhagen1, R. Zeilinger2
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Objectives: Circulating tumor cells (CTCs) are present early in the metastatic process, and their detection has been implicated as an independent diagnostic marker for residual disease. In addition, the isolation of pure CTCs is of interest for further characterization using molecular biology techniques and the development of individualized therapy.

Materials and Methods: Leukapheresis was used to enrich leukocytes from healthy donors. To meet the elutriation requirement of 10⁶ monocytes, the entire blood volume of donors was circulated twice (10L). Ovarian epithelial CaOv-3 tumor cells were labelled with carboxyfluorescein succinimidyl ester (CFSE) and spiked into the apheresate at 2.6 or 26 tumor cells in 1x10⁴ leukocytes. The elutriation system was programmed to enrich monocytes providing 5 distinct leukocyte fractions. The leukocyte distribution within the apheresate and elutriation fractions was quantified using flow cytometry TruCOUNT™ tubes and an antibody cocktail containing anti-CD45, CD15, CD56, CD3, CD19 and CD14. Tumor cells were characterized as CFSE+/EpCAM+/CD45- events. Magnetic beads coupled with EpCAM via a DNA linker were further used to isolate and purify tumor cells from the elutriation fractions. Recovery was confirmed by immunofluorescence.

Results: The recovery of spiked tumor cells after elutriation was consistently above 60%. 63.6±18.6% (n=4) of the recovered tumor cells were found in the last elutriation fraction resulting in an enrichment factor of 7.3 (n=2) for antibody beads. The recovery of tumor cells was not significantly changed by co-adsorption of the other cytokines or platelets. The purity of recovered tumor cells, determined by immunofluorescent counting, was 51% in fraction 5, which consists of enriched monocytes.

Conclusions: Our data demonstrate that CTCs can be enriched from a large blood volume using elutriation procedures provided with magnetic beads. Specific beads tumor cells may be isolated in high purity. Tumor cell recovery may be further improved by substituting bead isolation with flow cytometry cell sorting.

O109 WITHDRAWN

O110 MODELING CYTOKINE CAPTURE IN A HEMOADSORPTION DEVICE FOR THE TREATMENT OF SEPSIS M.V. DiLeo1,2, J.D. Kimmel3, J.A. Kellum2, W.J. Federerspiel1,2,5
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Objectives: Sepsis, systemic inflammation in the presence of infection, is characterized by high concentrations of inflammatory mediators called cytokines. Extracorporeal cytokine adsorption using microporous sorbent beads has been shown to us to decrease circulating levels of cytokines and increase survival time in rats. The objective of this study was to develop and validate a predictive mathematical model describing the dynamics of cytokine removal by our hemoadsorption device.

Materials and Methods: Multiscale perturbation analysis was used to develop a simple analytical expression for cytokine removal rate that accounts for diffusion and adsorption within each polymer bead and convection through the device. In vitro cytokine capture was performed by spiking IL-6, TNF, and IL-10 in horse serum, circulating the solution through a 1 mL cartridge of sorbent beads, and measuring cytokine removal rate. Cytokine adsorption within single beads was investigated by quantifying intraparticle intensity profiles at specific time points using fluorophore-labeled cytokines and confocal laser scanning microscopy. The model was fit to cytokine adsorption profiles using nonlinear regression.

Results: Recirculation and CLSM data were fit well to the model (R²>0.92 and 0.98, respectively). The model was able to predict changes in removal rate associated with changes in initial cytokine concentration, plasma flow rate, device, bead volume and bead level and size. As predicted by the model, individual cytokine removal rates were not significantly changed by co-adsorption of the other cytokines or of h2-microglobulin. The CLSM data confirmed that only the outer ~20% of beads was investigated by quantifying intraparticle intensity profiles at specific time points using fluorophore-labeled cytokines and confocal laser scanning microscopy. The model was fit to cytokine adsorption profiles using nonlinear regression.

Results: Circulating tumor cells (CTCs) are present early in the metastatic process, and their detection has been implicated as an independent diagnostic marker for residual disease. In addition, the isolation of pure CTCs

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**O111**

**Optimized Biocompatibility and Performance of a New Steam Sterilized Plasma Separation Membrane**


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**Objectives:** Plasma exchange has become a therapeutic option for various immunologic and metabolic disorders. In addition, the application of cascade techniques with re-infusion of plasma gains more importance. However most plasma separation membranes have been developed decades ago and may exert unfavourable properties concerning membrane characteristics, biocompatibility and sterilization techniques. Therefore we evaluated a new plasma separation membrane, Plasmylane 6, which was manufactured by state of the art membrane and device technology.

**Materials and Methods:** The differences between the newly developed plasma separations filter Plasmylane 6 vs PF200N are the wall thickness (50µm vs 150µm), the total surface (0.6m² vs 0.35m²), the sterilization procedure (steam vs ETO) and the material (PAES vs PP). Plasmylane 6 has a smoother inner surface than PF200N shown by SEM. The separation layer is closed to the inner surface to reduce blocking of pores. In an open-label, prospective study, 8 pts with various indications for therapeutic plasma exchange were enrolled to evaluate efficacy and biocompatibility of Plasmylane 6 vs PF200N.

**Results:** Plasmylane 6 was well tolerated in a heterogeneous pts group. We detected significantly improved biocompatibility for Plasmylane 6 as assessed by complement activation (TCC, C3a) and leukocyte drop. No clinically significant platelet drop was observed for both filters. Plasmylane 6 showed higher levels of markers for glomerulonephritis and total protein comparable to PF200N. Priming duration was significantly lower and no foam was observed as compared to PF200N.

**Conclusions:** Steam sterilized Plasmylane 6 exerts improved performance and biocompatibility, particularly important for its use in cascade filtration and other therapies with re-infusion of treated plasma.

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**O112**

**The "MARS 500" Project: Bioimpedance Analyses of Body Water, Fat and Muscle Mass Under Controlled Conditions**

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**Objectives:** Bioimpedance measurements are increasingly applied for the determination of body composition in terms of water, fat and muscle mass. Continuous analyses under controlled conditions of food intake and exercise are rare and difficult to perform. However, such bioimpedance analyses would yield reliable information on the impact of food intake and exercise on changes of the above physiological parameters.

**Materials and Methods:** The MARS 500 Project is performed under the control of the Russian Institute of Biomedical Problems (IBMP). The overall aim of the project is to simulate the flight to the planet Mars in a terrestrial module which provides conditions of 200 m² for a living room, a kitchen, a medical part and an area for research. Facilities are attached to a warehouse containing food, drinks and expandable material for scientific experiments. Six healthy volunteers spend an initial period of 105 days followed by a second period of more than 500 days in a metabolic ward under controlled, apart from the lack of zero gravity and radiation, realistic space conditions. Food intake is carefully monitored and biophysical experiments are performed. Bioimpedance analyses are done weekly. Thus, data on diet changes and exercise are obtained in a close follow-up. Further analyses on body liquids, such as blood, sweat and urine are documented.

**Results:** Since March 31, six healthy volunteers have started the "Flight-to-Mars-simulation" experiment in 2009. They stay in the metabolic ward for a primary period of 105 days. Urine, sweat and blood samples are analyzed and bioimpedance measurements performed.

**Conclusions:** The MARS500 mission allows performing bioimpedance analyses of healthy volunteers, living in a metabolic ward under conditions close to space reality. These analyses yield unique information on changes of body composition under the extreme conditions of an isolated space ship.
Abstracts: XXXVI Annual ESAO Congress, 2-5 September 2009, Compiègne - France

Conclusions: The new model correctly predicts water absorption and albumin transport although the clearance of SA from blood to the PC is lower than reported in clinical studies. The impact of vasomotion, for the first time taken into account in a model of the water and protein transport and distribution in the tissue, has rather local character.

O115
VIRTUAL PATIENT SPIROMETRY SYSTEM AVAILABLE VIA INTERNET
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Objectives: Recently, computer methods in education are of increasing significance. A virtual patient (VP) may be an example. The VP is easy to duplicate, and thus each student may have his/her own patient and can choose a convenient place and time for learning. There are no ethical, financial or legal problems when a student makes a mistake, even if he/she “kills” the VP. Instructors can simulate at each moment a disease that they want to discuss.

Materials and Methods: A virtual respiratory system (VRS), previously elaborated, was supplemented with a user interface. This made possible to create the Tgol.e-spirometry™ system, simulating forced expiration in various conditions in a user friendly way.

Results: Equations of the original VRS were changed to make it possible to interpret and name physiologically those of equations parameters, which are enabled to be set by users. The values of such parameters are expressed by percentages of some reference values. Thus, users do not need to know mathematics at all. The settings might be changed globally or locally: five bronchi features (obstructive diseases) as well as the lungs size and parenchymal compliance (restrictive diseases). Several other parameters, such as deepness of the forced inspiration or expiration, can be changed too. Then, influence of such changes on forced spirometry results (the flow-volume curve and numerical indices) can be analyzed. Courses of other variables, e.g. instantaneous airway resistance, can be also presented. The system is available via Internet from the website www.virtual-spirometry.eu

The system may have several educational applications, such as the following, already existing ones: (a) its use can be incorporated in teaching curriculum to medical students; (b) an instructor can illustrate his/her own lecture with simulations.

Conclusions: It is possible to present a very complex model in such a way that the model may be useful for wide medical audience.