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Artificial Kidney - Uremic Toxins - *SYMPOSIUM*

K1 (EI0154)

AGES IN HEMODIALYSIS: TISSUE- AND PLASMA- AUTOFLUORESCENCE

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Objectives: Advanced glycation end-products (AGEs) accumulate in all human subjects, e.g. in the skin. Because part of the AGEs shows autofluorescence, skin autofluorescence (SAF) generally increases with calendar age. SAF values above normal have been reported in patients with cardiovascular (CV) risk. SAF was a strong marker of CV mortality in hemodialysis (HD) patients [Meerwaldt, JASN 2005]. High levels of plasma AGEs were reported as well in HD patients compared to control subjects [Floridi, NDT 2002]. A consortium was formed to study the use of SAF for measuring the effectiveness of interventions that aim to decrease (CV) risk in dialysis patients.

Methods: SAF was measured non-invasively with the AGE Reader (DiagnOptics Technologies B.V., Groningen, The Netherlands) at the inner forearm in HD patients: 33 (Umeå), 170 (Skopje), and 109 (Groningen). In Umeå and Skopje measurements were repeated at least twice a year for 15 resp. 24 months. In Umeå and Groningen measurements were performed before and after dialysis; plasma autofluorescence was determined in these patients before and after dialysis as well.

Results: Plasma fluorescence decreased 12% ($p<0.001$) during a dialysis session, whereas SAF did not change significantly. This confirms that SAF mainly represents tissue fluorescence. In all centers mean SAF was increased 40-60% as compared to healthy subjects. The yearly increase of SAF in Skopje was higher than in Umeå, and about ten times higher than in healthy subjects. Some variation in SAF during the year was observed in the Umeå data, which needs further investigation.

Conclusions: High concentrations of plasma AGEs seem related to their increased accumulation in tissue, as are visible in the increased skin autofluorescence and rate of increase. By not being influenced by single dialysis sessions, SAF shows to be a useful marker for assessing AGE accumulation and studying the related CV risk in HD patients.

O1 (EI0222)

THE USE OF A SKIN AGE READER TO EVALUATE RISK OF CVD AND MORTALITY IN DIALYSIS PATIENTS

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Objectives: To measure annual increase in Skin Autofluorescence (AF), a marker of accumulation of Advanced glycation end-products (AGEs) in the skin of hemodialysis (HD) patients and various plasma markers including Heart-type Fatty acid-binding protein (H-FABP) in order to find factors that can predict the mortality of HD patients.

Materials and Methods: 169 HD patients were enrolled in a clinical prospective study. Skin AF was measured at 4 time points at approximately 6 months intervals. At the same time points the routine blood chemistry and plasma markers of oxidative stress (Superoxide dismutase and Myeloperoxydase), inflammation (C-reactive protein: CRP), endothelial activation (Inter-cellular adhesion molecule-1: ICAM-1 and von Willebrand Factor) and myocardial and kidney damage (H-FABP) were measured. The entire study lasted 32 months.

Results: Skin AF was increased in HD patients, especially in those with diabetes, in which it showed to be the sole independent marker of the presence of cardiovascular diseases (CVD). The mean annual increase of Skin AF (ΔAF) was 0.15±0.09 AU (mean±standard error). Seasonal fluctuations in Skin AF with a mean of 0.31±0.10 AU (mean±standard error) were only present in patients with Hepatitis C. In the multivariate Cox regression analysis we found that age diabetes, hypertension, annual ΔAF and values of CRP, ICAM-1 and H-FABP at the start of the study were independent predictors of overall mortality. Strong predictors of CVD mortality were age, diabetes, male gender, annual ΔAF, and H-FABP and albumins. Moreover, combined use of annual ΔAF and single measurement H-FABP gives even better results in the prediction of the CVD mortality risk than separate use.

Conclusions: Annual ΔAF and single measurement of H-FABP are strong independent predictors of overall and CVD mortality in HD patients.

O2 (EI0191)

DOES THE ADVANCED GLYCATION END-PRODUCTS (AGES) FOOD INTAKE INFLUENCE MORTALITY IN DIALYSIS PATIENTS?

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Objectives: The diet is a source of AGEs. Nutrition recommendations on calories and protein intake for patients on chronic maintenance hemodialysis are already published. Data about the influence of the ingested amounts of AGEs on the mortality is still unavailable. Aim of study: to assess the impact of calories, protein and AGEs intake on mortality in dialysis patients.

Methods: 150 patients (mean age: 55.69±13.5 years, dialysis vintage 8.9±6.6 years) were included in a prospective study. Patients were followed for 36 months, up until death, kidney transplantation or until the end of the observational period. Dietary records for 7 days were obtained and calories and protein intake were calculated. Daily AGEs intake was estimated by Teresia Goldbaerg et al. (J Am Diet Assoc. 2004). AGE intakes of survived and deceased patients were compared with independent T- test.

Results: In the time period of 36 months of follow-up 36(23%) HD patients died and one was kidney transplanted. AGE food intakes did not differ between the two groups of survived and died patients (9.3±4.2 vs. 9.2±3.6 MU/day, $p=0.868$). We found borderline significance for the difference in calories intakes, in favor of the survived patients (31.2±8.0 vs. 27.8±5.8 Kcal/kg/day, $p=0.061$). The two groups also did not differ in the protein intake (1.31±0.73 vs. 1.10±0.50 g/kg/day, $p= 0.240$).

Conclusions: Mortality of dialysis patients is probably affected by more powerful factors than AGE food intake. Further studies are needed to confirm the impact of all nutritional factors on the survival.

O3 (EI0192)

SKIN AF AND FOOD, IS THERE ANY RELATION?

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Objectives: To investigate the influence of nutrition, especially Advanced glycation end-products (AGEs) intake, on the accumulation of AGEs in Hemodialysis (HD) patients.

Methods: 156 HD patients were enrolled in this study. Skin Autofluorescence (AF) was used to measure the AGEs accumulation. The enrolled HD patients were asked to record their daily food intake over a period of one week. From these recordings daily calorie, protein and AGEs intake were calculated. The Body mass index (BMI), as a measure of nutritional state, was calculated. The routine blood chemistry and plasma markers of oxidative stress (Superoxide dismutase and Myeloperoxydase-MPO), inflammation (C-reactive protein-CRP), endothelial activation (Inter-cellular adhesion molecule-1 and von Willebrand Factor) and myocardial and kidney damage were measured.

Results: The mean protein, calorie and AGEs intake correlated with each other ($R=0.56$ $p<0.01$; $R=0.36$ $p<0.01$; $R=0.33$ $p<0.01$). We found that the AGEs and protein intake is highest in the third quintile (BMI 21.16-23.79 kg/m^2) whereas the calorie intake was highest at the fourth quintile (BMI 23.79-27.00 kg/m^2). In the multivariate analysis we assessed the contributors of the annual increase of Skin AF. The independent contributors of the increase of the Skin AF were: lower or higher BMI than the 4th quintile of BMI (23.79-27.00 kg/m^2), lower AGE intake as well as shorter HD vintage and higher annual increase of MPO. We analyzed the influence of BMI on the annual increase of Skin AF and we found that the relationship between BMI and the annual change Skin AF can be represented as a U-shaped curve. The lowest point of the U-shape curve is 31 kg/m^2.

Conclusions: We found that being slightly overweight and having higher AGEs intake result in lower AGEs tissue accumulation. The correlation between the change of Skin AF and BMI is a U-shaped curve with a bottom at 31 kg/m^2.

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O4 (EI0409)

HOW CAN WE OPTIMIZE HEMODIALYSIS TO PREVENT FROM AGES

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Objectives: Glucose degradation products are produced progressively during the lifespan. As mentioned above, this results in an accumulation over time. Such accumulation is best known by patients that suffer from diabetes mellitus. The aim was to investigate if skin-AF and plasma-AF were influenced by hemodialysis (HD).
Methods: Design: clinical prospective studies of hemodialysis patients. The first study was performed to investigate if hemodialysis may influence AGEs accumulation in dialysis patients in general. Since skin-AF is a good representative for GDP accumulation in these patients.
The patients included were on chronic HD. Glucose 5mmol/L was used as part of the dialysis fluid. Various dialysers were used. More than 30 patients were included. Paired statistical analyses were performed. The power of the studies is estimated to be more than 80%. Skin AF was measured before and after HD. Plasma fluorescence was also measured before the start and after HD.
Results: The results showed that HD resulted in a significant reduction of plasma AF. This resulted in a second study to evaluate if there was a difference in the efficacy using either high-flux dialyzers or low-flux dialyzers. The patients were randomized in a cross-over design using either HF or LF dialyzer. Skin and plasma AF was done as in the study above. The results showed no significant difference in skin-AF, using LF versus HF.
Conclusions: This data show that HD patients had a significant elevated skin-AF. The GDPs, estimated by plasma and skin AF, will be reduced by HD. The molecules removed seem to be of a lower molecular size. Therefore sufficient dialysis is important to reduce accumulation of GDPs. The effect seems enough by using LF dialyzers in this regard. The effect of diet and glucose-free dialysates has to be further explored.

Smart and Responsive Biomaterials - *SYMPOSIUM*

K2 (EI0042)

SMART BIOMATERIALS

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Smart polymers are able to respond to changes in their environment. Shape-memory polymers are an example for stimuli-responsive materials, which can change their shape on demand. Such Polymers are of interest for a variety of application areas including biotechnology and medicine. Only a few polymeric biomaterials are established in clinical applications to date and most of these biomaterials have not primarily been developed for biomedical applications. Many implants have initially been developed to fulfill a structural/mechanical function. Examples for this category of medical devices are surgical sutures, hip prostheses or hernia meshes. The predictability of the long-term behavior of biomaterials in physiological environments became apparent as a major challenge. In this context, experimental as well as computational tools are being developed to evaluate the mechanisms of polymer degradation. With increasing clinical experience it became furthermore apparent that one single function is not sufficient, but multifunctionality is required. Vascular stents, which initially were purely metallic devices with a specific structural function, have been further developed by adding polymeric coatings to improve their hemocompatibility, which were partially loaded with drugs to avoid restenosis. Presently, degradable stents are under development. In this presentation, the scientific challenges of combining several functions in one material are described and examples for dual and triple functional polymers are given. Finally, the potential application of theses biomaterials in regenerative medicine is outlined. Potential applications include smart implants or drug release systems inducing endogenous regeneration and scaffolds for tissue engineering applications.

O5 (EI0354)

A COMPOUND NERVE GUIDE CONSTRUCT FOR PERIPHERAL NERVE REGENERATION

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Objectives: Currently, autologous nerve grafts are gold standard for repairing critical-size gap defects in peripheral nerves following accidents, injuries etc. However, this results in donor site morbidity and functional recovery is often unsatisfactory. Instead, one can use artificial conduits to guide nerve regeneration. The goal of this study is to develop such a construct to provide structural and biochemical cues promoting axonal growth.
Methods: The nerve guide consists of an inner chitosan core having axially directed, continuous pores made using directional solidification and an outer shell of electrospun polycaprolactone (PCL) fibres. Chitosan solution in CH_3COOH was filled into copper molds and cooled down with defined cooling rate and temperature gradient. This resulted in unidirectional growth of ice crystals along the temperature gradient, which were later removed using freeze-drying forming a porous structure. This was then mounted on rotating collector and a layer of PCL fibres was electrospun to give the final construct.
Results: The construct had a total diameter of 1.3mm. The chitosan core had a diameter of 0.7mm with a pore size of 40µm. The thickness of the electrospun wall was 0.3 mm with a fibre diameter of 3–4µm. Pore size of the chitosan core, as well as wall thickness and fibre diameter of the electrospun shell, can be tailored as required by changing the process parameters.
Conclusions: A compound construct was produced to act as a nerve guide conduit for repairing nerve defects. In future studies we will characterize its regeneration potential *in vitro* and *in vivo*. These studies will elucidate if the construct provides structural and biochemical cues to enhance peripheral nerve regeneration. This work is supported by funding from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) for the Cluster of Excellence REBIRTH (From Regenerative Biology to Reconstructive Therapy) and the International Foundation for Neurobionics (to KHT).

O6 (EI0404)

CHARACTERISING ADVANCED NANOPOROUS ACTIVATED MAST CARBONS FOR THE TREATMENT OF KIDNEY DISEASE

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Objectives: A range of medical grade, phenolic resin based, activated carbon adsorbents (ACs) have been developed which offer great potential for the removal of albumin bound and larger molecular weight biological toxins associated with the progression of kidney disease. MAST carbons combine the superior adsorptive capacity of ACs with uniquely tailored nanoporosity to augment the removal of uremic toxins, which are poorly removed by current hemoperfusion systems. A proof of concept study was carried out in order to assess the impact of MAST AC form and porosity on biocompatibility and functionality with respect to the removal of key biological toxins associated with kidney disease.
Methods: The physical properties of a range of MAST carbons in bead and monolithic form were assessed by scanning electron microscopy and porosimetry. Cell based assays were used to assess the cytotoxicity of carbon leachate. The removal capacity of the carbons for a range of uremic toxins associated with renal failure was assessed using spectrophotometric, HPLC and ELISA based analysis.
Results: MAST carbons have a high surface area for adsorption and were distinguished from other commercial carbons by the presence of larger nanoporous domains. MAST carbons were not cytotoxic and were capable of removing significant amounts of the larger biological toxins not removed by purely microporous carbons alone.
Conclusions: The surface structure and form of MAST ACs may be manipulated to produce a material which is highly suited to the removal of protein bound and high molecular weight biological toxins. In this way nanoporous MAST ACs may offer a therapeutic strategy to augment current hemodialysis-based systems by reducing uremic toxicity effects associated with disease progression.

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O7 (EI0276)

IN-SITU CAPTURE OF ENDOTHELIAL PROGENITOR CELLS (EPCS) ON VEGF-BOUND DEVICES: SURFACE ARCHITECTURE AND CELLULAR RESPONSES

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Objectives: This study focuses on 1) developing surface architecture enabling *in-situ* capturing of EPCs flowing in arterial bloodstream aiming at long-term non-thrombogenic potential of implanted devices (stent and small diameter artificial graft), 2) determining *ex vivo* cellular responses including selective harvesting, cell adhesion and proliferation and shear-stress resistance potentials and activation of intracellular signal transduction pathways, and 3) reporting preliminary *in vivo* cellular responses in porcine models.
Methods: 1) Covalent bonding of molecules [vascular endothelial growth factor(VEGF) and two VEGF receptor antibodies and Tie-1 and -2 antibodies] on thin-layered vinyl alcohol-copolymer. 2) Culture of human mononuclear cells on these protein-bound substrates and histocytochemical analyses, 3) determination of hydrodynamic shear stress dependence of adhered EPCs and endothelial cells (ECs) by radial flow chamber technique, 4) implantation of stent and electrospun artificial graft in porcine models.
Results: Proteins were covalently bound to the polymer surface via activation of hydroxyl group. Among molecules examined, only VEGF exhibited high adhesion and proliferation characteristics similar to those of fibronectin, and a quite high differentiation potential (expression of surface markers specific for EC) with culture time. In addition, day-order continuous activation of intracellular transduction pathways (phosphorylation of VEGF receptor, FAK, ERK and Akt) was observed for ECs adhered on VEGF-bound substrate. Once adhered, high detachment resistance to laminar flow was observed under arterial shear stresses. Based on these results, preliminary implantation study in porcine models using ultrasonic-atomized stents and custom-design electrospun artificial grafts, both of which are surface-architectured with bound VEGF, was conducted. The results showed that cells adhered on blood-contacting surface expressed VEGF receptor.
Conclusions: The target molecule defined in this study is VEGF. Surface-bound VEGF expresses high adhesion, proliferation, hydrodynamic shear stress resistance and differentiation potentials. Simple but reliable surface fabrication technology may provide non-thrombogenic potential to implantable cardiovascular devices.

O8 (EI0111)

RESILIENT AMORPHOUS NETWORKS WITH SHAPE MEMORY PROPERTIES FOR USE IN MEDICAL APPLICATIONS AND TISSUE ENGINEERING

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Objectives: In tissue engineering, flexible, form-stable and resorbable elastomeric networks can be used to prepare scaffolding structures with most advantageous properties. Here we describe the synthesis and characterization of a series of amorphous photo-crosslinked networks with tunable thermal- and mechanical properties based on trimethylene carbonate and D,L-lactide.
Methods: The co-oligomers were synthesized by ring opening polymerization of the corresponding monomers in the presence of varying amounts of hexanediol, and then functionalized by methacrylation. Networks were obtained by UV crosslinking in the presence of a photoinitiator. Of the obtained crosslinked structures, the network properties and the thermal- and mechanical properties were assessed. The shape recovery behavior of the different networks was evaluated quantitatively.
Results: Amorphous networks were prepared from macromers with different molecular weights in which the monomer molar ratios were varied between 0:1 and 1:0. This allowed tuning of the glass transition temperature and mechanical properties. The values of the toughness, ultimate tensile strength and elongation at break of flexible networks with glass transition temperatures above room temperature increased with increasing macromer molecular weights. Networks prepared from macromers with a TMC content of 0.4 to 0.6 had Tg values close to or below body temperature. These networks are especially interesting as they are relatively rigid at room temperature, and flexible with mechanical properties in the range of soft tissues at body temperature. From these materials, porous and non-porous devices were fabricated. At room temperature these devices are relatively rigid and can be implanted non-invasively in their temporary shape, while at body temperature they return to their original permanent shape to perform a desired function.
Conclusions: These properties, and their biodegradability and elasticity, make these networks materials very well-suited for the preparation of self-deploying implants in medical applications like tissue engineering, drug delivery, stenting and the support of soft tissues.

CARDIOVASCULAR GENERAL 1: DEVICES – *GENERAL SESSION*

O9 (EI0172)

ONE-YEAR COST COMPARISON BETWEEN CARDIAC TRANSPLANTATION AND LVAD THERAPY

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Objectives: Left ventricular assist devices (LVADs) are increasingly used in end-stage heart failure, not only as bridging therapy but also more as destination therapy. The perceived cost of LVAD therapy is considered to be a limit in its application.
Methods: Actual hospital invoices of 20 consecutive surviving transplant patients (HTX) and 21 consecutive surviving patients receiving an LVAD were reviewed. All in hospital costs starting from the date of operation until one year post discharge were collected. Hospital costs were defined as the sum of all reimbursed costs and the patient's own share. The cost of the donor heart was calculated as an average of the total costs of explantation and transportation fees. The ambulatory cost is a sum of the actual hospital invoices and the mean ambulatory drug cost per month.
Results: The duration of the first hospitalization was significantly longer in patients receiving an LVAD (LVAD-Group: 44.9±24.6 days versus HTX-Group: 24.5±7.3 days; $p<0.01$). The initial hospitalization cost was higher in the LVAD-group (LVAD-Group: €40793±19660 vs. HTX-Group: €27439±13889; <0.05). The cost of the device was €69239 versus €7810 for the donor heart. Monthly ambulatory costs were higher in the HTX-Group (HTX-Group: €3036±815 vs. LVAD-Group: €699±598; $p<0.001$). The overall cost after one year was higher in the LVAD-Group (LVAD-Group: €118420±18791 vs. HTX-Group: €72935±15253; $p<0.001$)
Conclusions: LVAD therapy is initially more expensive than heart transplantation, predominantly by the device cost. However, the ambulatory cost of a transplanted patient is significantly higher per month, due to the need for immunosuppressive medication and its monitoring. Given that these higher monthly costs are fixed, in the long term LVAD therapy will become less expensive compared to transplantation.

010 (EI0428)

IMPLANTABLE BIVENTRICULAR ASSIST DEVICE: FIRST *IN VIVO* RESULTS

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Objectives: Right heart failure is a common complication in patients with LVAD therapy. To assist both ventricles with one device, a completely implantable, pulsatile BVAD with specific assistance for the left and right heart is to be developed.
Methods: The compact device includes two pump chambers that are alternately compressed by hydraulic fluid, using a high efficient electro-hydraulic energy converter. Hydraulic bearings enable enhanced lifetime of the gear. The flat design (Vol. 435cc) allows for completely implantation. Differentiated ejection and acute control of the stroke volume are enabled by a control algorithm, monitoring the filling of the pump chambers and the heart frequency. New seamless pump chambers are optimized by CFX and flow measurements using non-newtonian fluid. The surfaces of the pump chambers are textured to allow cell adhesion. The TET is verified in vitro, enabling complete implantation.
Results: The performance and durability of the BVAD was tested in mock loops up to 145 days. With a frequency of 120bpm, the BVAD generates 5.1L/min output for each ventricle. A speed of 6000 rpm of the drive unit enables the maximum pump frequency of 180 bpm with a maximum flow of 7 L/min. The energy consumption of the pump is between 6 and 9 Watt. A nearly physiological flow field is generated in the new chambers. During filling phase, two recirculation zones similar to those found in the human left ventricle are

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observed. Increased shear rates up to 1500 1/s are observed downstream the inflow. No superior warming of the drive unit was measured.
Conclusions: The developed BVAD combines the advantages of displacement pumps and rotary blood pumps to support patients up to a BMA of 1.8 m^2. Separate control of the stroke volume and triggering the BVAD's frequency to the heart should enable sufficient unload of the ventricles.

O11 (EI0131)
EVALUATION OF THE MAGLEV MOTOR AND DESIGN OF THE CENTRIFUGAL BLOOD PUMP FOR PEDIATRIC ARTIFICIAL HEART
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Objectives: We have developed a Maglev artificial heart for the use in infant patients, which require small size compared to adult devices. The active magnetic bearing offers better biocompatibility and longer device lifetime than the artificial heart by eliminating any actual physical contact. In this paper, a miniaturized self-bearing motor and centrifugal blood pump for pediatric artificial heart are reported.
Methods: The self-bearing motor consists of a top stator, a bottom stator and a levitated impeller set between both stators, which have identical structure. The impeller is suspended axially with a double stator mechanism to enhance a motor torque with smaller device size. The motor regulates an axial position and a rotating speed of the impeller by using vector control algorithm. A target pump performance of the pediatric artificial heart is set as a flow rate of 1 L/min against a head pressure of 100 mmHg. The pump has been designed with the computational fluid dynamics simulation.
Results: The diameter and height of the developed motor are 24 mm and 43 mm, respectively. The volume of the artificial heart is 21 mL. The motor can produce an attractive force of 16 N with an air-gap of 1.5 mm, and a rotating torque of 13 mNm with the impeller speed of 4000 rpm. From the simulation results, an impeller speed of 4000 rpm is required to achieve the target head pressure and the flow rate. At this time, an axial thrust force of 0.14 N and a torque of 3.6 mNm act the levitated impeller.
Conclusions: The large attractive force and the rotating torque indicate enhanced magnetic suspension and rotation performance for a smaller size and larger air-gap. This developed Maglev motor is suitable for the use in the pediatric artificial heart.

O12 (EI0122)
COMPARISON OF 50CC PENN STATE VAD DESIGNS USING PARTICLE IMAGE VELOCIMETRY
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Objectives: Congestive heart failure remains a major cause of death worldwide. We continue to develop and study ventricular assist devices (VADs) in an effort to assist these patients. As these devices miniaturize, thrombosis remains a major concern. Our current focus is to compare two pulsatile VAD designs using *in vitro* optical measurments to focus on the local wall shear rates in areas that may be prone to thrombus deposition. Furthermore, we compare the effect of heart rate variabililty on the local flow and its potential impact on said deposition.
Materials and Methods: To measure and calculate the local wall shear rates in two acrylic model 50 cc VAD designs, particle image velocimetry (PIV) was used for heart rates from 75-150 beats per minute and the appropriate systolic duration. A standard mock circulatory loop and blood analog were used to simulate the cardiovascular system. Magnification of 12 microns per pixel was achieved for the PIV system and an error analysis performed. Multiple planes of PIV data were collected and wall shear rate maps produced.
Results: The flow patterns for each device were not significantly altered when the heart rate increased. The major effect was an increase in velocity and subsequently, wall shear rate. Roughly speaking, the magnitudes of the shear rate scale by approximately the square of the inlet velocity. There were some differences near the front wall of the device, which was the major geometric difference between the two VAD designs.
Conclusions: The local fluid dynamics plays a significant role in the development of thrombus on a surface. To facilitate the success of these VADs, measurements were taken for varying heart rates as would be seen clinically. A new wall shear rate correlation coefficient has been developed to correlate this variability to potential deposition.

O13 (EI0089)
HEMODYNAMICS OF A VALVELESS COUNTERPULSATION HEART-ASSIST DEVICE: LASER DOPPLER VELOCIMETRY AND COMPUTATIONAL FLUID DYNAMICS
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Objectives: Single port valveless pneumatic counterpulsations heart-assist devices have had, normally, a high incidence of thrombus formation due to blood stagnation regions in the blood chamber. This prevents a success of these device types in a long-term application. Blood chamber flow of a novel 32 mL stroke volume blood chamber design was investigated.
Methods: To investigate blood washout behavior of the new design of a valveless counterpulsation device (CPD) with disk-shaped blood chamber (30 mm radius and 25 mm maximal thickness), Laser Doppler velocimetry (LDV) and Computational fluid dynamics (CFD) were applied. Simplified static CFD model using flow solver FLUENT (ANSYS Inc., USA) was used to visualize 3D flow structure at the end-filling phase. The time resolved flow investigation of the CPD chamber and the inlet port was done by two-component LDV device (Dantec Dynamics, Denmark).
Results and Discussion: Flow investigations found that tangentially designed CPD inlet 10mm diameter port forms during a filling phase a strong, in general two-dimensional (2D) moving vortex fully filling the blood chamber. Such vortex is considered to be indicative for a good washing. No regions of persistent blood stagnation or recirculation bubbles were observed. Laminar shear stresses estimated by CFD were well below the known hemolysis threshold of 400 Pa inside the blood chamber. The short curved graft generated the helical flow pattern forming a minor secondary flow (helicity) of the 2D vortex. This secondary flow is considered to be favorable for the washing of the region between the membrane and the chamber housing.
Conclusions: The CPD blood chamber flow has good washing characteristics without stable areas of blood stagnation during the entire pump cycle, thus promising a low risk of thrombus formations.

O14 (EI0085)
COMPARISON OF THE FLOW PATTERN AND STAGNATION AREAS DEVELOPMENT IN THE VAD UNDER TRANSIENT AND STEADY STATE CONDITIONS
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Objectives: A numerical flow analysis and a comparison of operation of the pneumatic VAD of the POLVAD EXT™ developed in FCS, Zabrze, type under steady state and transient conditions are discussed. In the transient test, a motion of the diaphragm as well as discs are simulated. The valves are based on J. Moll's design, with later modifications introduced at the Institute of Turbomachinery, TU Lodz.
Methods: Flow simulations for two different approaches are compared. In the steady state, two opposite operational states, namely diastole and systole, are simulated. In order to fulfil code requirements in the steady state, both discs are open. For diastole, the inlet disc is fully open whereas the outlet one is almost closed. For systole, it is the opposite. In the transient test, four cycles of operation of the VAD are calculated to minimize an influence of initial conditions on the analyzed flow. A motion of the diaphragm and discs are forced by equations developed on the basis of the VAD normal operation. Discs are modelled as immersed bodies and operate in a full range, from open to closed state with respect to the diaphragm motion. The non-Newtonian blood model based on the Power Law is applied. Velocity, streamlines, pressure distributions in the region of investigations are presented and analyzed. The ANSYS CFX v.12.1 code is used to perform the numerical experiment.
Results: The numerical experiment conducted shows differences in flow patterns as the inertia of fluid particles plays a significant role in systole in the case of transient calculations.
Conclusions: The numerical study shows that steady state simulations are useful at the pre-design stage, as they need significantly smaller computational efforts. Nevertheless, the transient method with a moving membrane and discs allows to identify flow structures that cannot be visualized in the steady state.

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Ambulatory Blood Processing - *SYMPOSIUM*

O15 (EI0435)
DESIGN AND *IN VITRO* PERFORMANCE OF A WEARABLE ULTRAFILTRATOR
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Objectives: Current ESRD therapy removes catabolites but is least effective in maintaining euvolemia. Control of hypo- and hypertension requires frequent water removal from ESRD and CHF patients. An ambulatory ultrafiltrator to address these problems that is safe, effective, and convenient is the object of this work.
Methods: A two-stage plasma filter – ultrafiltrator – produces a plasma flow from unanticoagulated blood. It uses silicon microsieves and a micofluidic flow channel to achieve high plasma fluxes over a 30 cm^2 surface. The plasma stream is reduced in volume and returned to the patient. The device is intended to work continuously at 1 mL/min, equivalent to 10 kg/wk.
Results: Transport feasibility and blood compatibility have been demonstrated in the laboratory. Animal experiments are underway. Specialized components including pumps and highly miniaturized sensors and monitoring devices have been developed. Fundamental information about microporous sieving of blood under controlled microfluidic conditions has been obtained, although the thrust of this report will be on the practical system.
Conclusions: The feasibility of a wearable ultrafiltrator capable of supporting euvolemia in ESRD and CHF patients that is safe, effective and convenient has been developed to the point of advanced animal testing.

O16 (EI0335)
BLOOD ACCESS FOR WEARABLE DEVICES: CATHETERS AND ACCESS PORTS
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Objectives: Wearable devices for hemodialysis and ultrafiltration are currently developed employing readily available technology but also novel concepts for pumps, dialysers and dialysate regeneration. The problem of blood access for these devices has not been widely discussed so far. Patient questionnaires regarding acceptance of home hemodialysis show that patients are afraid of fistulas and grafts but also of catheters because of safety aspects and difficulties to self-access, and because of the fear of complications. This paper discusses possible blood access concepts for wearable devices.
Methods: The investigation starts with the assumption that blood flows up to 100 mL/min are required to achieve a creatinine clearance of >30 mL/min which corresponds to kidney failure class 3 (moderate). For permanently connected wearable devices the long tunneled catheter may be the optimal blood access. For patients who want to disconnect temporarily, as well as for home-hemodialysis patients, implantable ports may be the better alternative.
Results: Based on the experience with an implantable access port several years ago we have designed an access port system that incorporates the following features: easy self-accessing with no bleeding or pain, fail safe flow shutoff in case of disconnection, novel transcutaneous tissue tract guide and infection prophylaxis when disconnected using an antimicrobial tixotropic gel.
Conclusions: The envisaged blood access port will reduce risks and complications of blood access for wearable devices and home-hemodialysis patients considerably.

O17 (EI0400)
NEPHRON+ WEARABLE ARTIFICIAL KIDNEY
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Objectives: Improving the health condition of renal patients via a wearable system for continuous blood purification.
Methods: A wearable artificial kidney is being developed embedded in an ICT-environment for personalised use and remote surveillance and control. The wearable device makes use of nanomaterials for sorption filtering in combination with miniaturized sensors and actuators. This allows for a small and wearable device.
Results: The basic design has been finished and the system is currently in the engineering phase. Some components and early prototypes have been tested, including first animal trials.
Conclusions: The Nephron+ consortium is making rapid progress and is well underway to meet its goals.

O18 (EI0114)
ATP-ADENOSINE-GLUTATHIONE CROSS-LINKED HEMOGLOBIN AS BLOOD SUBSTITUTE
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Objectives: Although Hb-based blood substitutes may offer a solution to transfusion medicine problems such as blood shortages, transmission of bloodborne pathogens and the RBC storage lesion, all commercialization attempts to date have been unsuccessful due to efficacy or toxicity issues. We have developed HemoTech, the next generation blood substitute that utilizes the concept of "pharmacologic cross-linking".
Methods: HemoTech, which consists of bovine Hb cross-linked intramolecularly with *o*-ATP and intermolecularly with *o*-adenosine, and conjugated with reduced glutathione (GSH), has entered the regulatory process in the USA. Several mandated requirements have been met including viral and prion clearance validation studies performed by BioReliance (Rockville, MD, USA) and various non-clinical pharmacology, toxicology, genotoxicity and efficacy tests conducted at the Research Toxicology Centre (Pomezia, Italy). The effects of HemoTech on appropriate physiological measures in human cell systems, normal animals and disease models have also been determined. The clinical proof-of-concept was carried out by the Instituto Sierovaccinogeno Italiano (S. Antimo, Italy).
Results: In this composition, while ATP prevents Hb dimerization, adenosine permits the formation of homogeneous polymers and counteracts the vasoconstrictive and pro-inflammatory properties of Hb via stimulation of adenosine A2 and A3 receptors. GSH introduces electronegative charge onto the Hb surface that blocks Hb's transglomerular and transendothelial passage and shields heme from nitric oxide and reactive oxygen species, thus enhancing vasodilation and lowering Hb's pro-oxidative potential. The results of preclinical and clinical studies indicate that HemoTech can work as a physiological oxygen carrier with prolonged intravascular persistence and produces no adverse nephrotoxic, neurotoxic, oxidative, or inflammatory reactions. It has vasodilatory activity and can reduce the vasoconstriction that follows hemorrhage as well as possesses high erythropoietic potential.
Conclusions: The obtained results confirmed that "pharmacologic cross-linking" of Hb molecules with ATP, adenosine and GSH is highly effective in designing a viable blood substitute.

Animal Models for Tissue Engineering - *SYMPOSIUM*

K3 (EI0423)
ANIMAL MODELS FOR OSTEO- AND CHONDROENGINEERING
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It is important to test tissue engineering approaches before they are transferred to the clinical arena. In vivo studies are necessary and demanded by federal institutions. The studies have to be performed according to animal welfare guidelines causing as least suffering as possible. For bone regeneration, several models are available. First, a rat femoral drill hole model is applicable for screening purposes. Thereafter, the constructs can be applied in a rat non-union femur defect model. The constructs are administered and tested by several imaging modalities. In the last phase, a sheep tibia segmental defect model with nail osteosynthesis can be used. The defect can be filled with constructs and healing can be assessed by µCT and histology. For chondroengineering a "humanized" nude mouse model is available. In the middle of the cartilage of human osteochondral discs, a defect is induced that can be filled with constructs. Subsequently, they are implanted subcutaneously in the back of nude mice. Thereby, an in vivo environment for the human construct is available. When constructs are proven to be effective in this model, a large animal model

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in minipigs can be employed. A chondral or osteochondral defect of different sizes can be made in the femur condyle of minipigs through mini arthrotomy. Subsequently, the defects can be treated with constructs. The faith of stem cells can be traced by a Xenogen camera. For this technique the cells are labelled with the luciferase gene. Upon injection of luciferin, photons can be visualized and measured by the Xenogen device. It is important to choose the optimal animal model for the hypothesis tested. It is also important to use sophisticated methods to obtain good quality data. Furthermore, one should have experience in applying the animal models to obtain consistent and reliable data.

O19 (EI0422)

GENE EXPRESSION AND CELL DIFFERENTIATION IN MATRIX-ASSOCIATED CHONDROCYTE TRANSPLANTATION GRAFTS: A COMPARATIVE STUDY

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Objective: Although scaffold composition and architecture are considered to be important parameters for tissue engineering, their influence on gene expression and cell differentiation is rarely investigated in scaffolds used for matrix-associated autologous chondrocyte transplantation (MACT). After testing the efficiency of cell-graft systems with very different scaffold characteristics for the treatment of cartilage defects in a horse model, we have comparatively analyzed the gene expression of important chondrogenic markers in four clinical applied transplant types.

Methods: Residuals (n=165) of four different transplant types (MACI®, Hyalograft®C, CaReS® and Novocart®3D) were collected during surgery and analyzed for Col1, Col2, aggrecan, versican, MIA and IL-1β by real-time PCR. Scaffold and cell morphology were evaluated by histology and electron microscopy.

Results: Despite the cultivation on 3D scaffolds, the cell differentiation on all transplant types did not reach the levels of native cartilage. Gene expression highly differed between the transplant types. The highest differentiation of cells (Col2/Col1 ratio) was found in CaReS®, followed by Novocart®3D, Hyalograft®C and MACI®. IL-1β expression also exhibited high differences between the scaffolds showing low expression levels in Novocart®3D and CaReS® and higher expression levels in MACI® and Hyalograft®C.

Conclusions: Our data indicate that scaffold characteristics as well as culture conditions highly influence gene expression in cartilage transplants and that these parameters may have profound impact on the tissue regeneration after MACT.

O20 (EI0421)

HUMAN PLACENTAL ALKALINE PHOSPHATASE TRANSGENIC ANIMALS AS A NEW TOOL FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE

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Mesenchymal stem cells differentiation capacity, production of cytokines, and immunosuppressive potential undoubtedly offer many therapeutic advantages. To further explore the therapeutic potential of regenerative treatments, it is necessary to trace the fate of individual donor or manipulated cells in the host organism. However, immune-mediated rejection of labeled cells is a general problem in transplantation studies using cells labeled with any immunogenic marker. Recently, we generated a novel *in vivo* cell tracking system consisting of a transgenic donor line ubiquitously expressing the heat stable enzyme human placental alkaline phosphatase (hPLAP). The corresponding transgenic recipient line expresses a heat labile mutant form of the enzyme (hPLAP^{E429G}). hPLAP^{E429G} differs just in one amino acid from the wild type form. Due to the slight alteration hPLAP is not recognized as foreign by the immune system of the hPLAP^{E429G} transgenic host. Nevertheless, the difference in heat resistance allows the identification of donor cells in histologic sections. To prove the utility of this system in regenerative medicine, we successfully isolated and characterized hPLAP-tg-MSCs from the bone marrow of hPLAP transgenic rats and mice. The cells were analyzed regarding the expression of stem cell surface markers, and differentiation potential. As a preliminary evaluation of their potentiality, we have evaluated their viability, proliferation and differentiation after seeding the cells in films, gels and scaffolding materials *in vitro*. The seeded cells on different biomaterials could be readily traced after 1, 3, 14 and 21 days of *in vitro* culture by hPLAP staining. As a proof-of-principle, we have injected bone marrow MSCs from hPLAP-tg rats *in vivo* into the knee joint of marker tolerant wild-type rats and found successful engraftment and differentiation of donor cells. In conclusion, this novel transgenic animal model may be a very useful tool to answer many open questions in MSC biology and regenerative medicine.

O21 (EI0424)

HOST RESPONSE TO BIOMATERIALS EVALUATED THROUGH DIFFERENT IMPLANTATION MODELS

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Objectives: The host response to a foreign body inherent to the biomaterials' implantation depends on both host and implanted material, particularly considering the host tissue diversity. The aim of this study was to compare the inflammatory response induced by implantation of starch-based scaffolds subcutaneous (SC) and intramuscular (IM) in rats.

Methods: Two methodologies, wet spinning (WS) and fibre bonding (FB), were used to prepare the scaffolds. The inflammatory response was assessed in male Sprague-Dawley rats (n=2), weighing 380-400g, 1 and 2 weeks post-implantation. In both models 4 scaffolds were implanted: SC through incisions in the dorsum and IM into the left and right scalenus dorsalis and gluteus muscles, respectively. The animals were kept in single-housed with food and water ad libitum and received analgesia in the first week. After each time period, each animal was anaesthetized with an intramuscular injection of ketamine/xylazine and sacrificed with an intracardial overdose. The scaffolds, surrounding tissue and nearby lymph nodes were explanted and used for histological analysis.

Results and Discussion: The WS and FB SPCL scaffolds did not elicit extensive leukocyte recruitment in both subcutaneous and intramuscular implantations in rats. The subcutaneous implantation induced a slightly higher inflammatory response as compared to the intramuscular implantation. However, in both situations the nearby lymph nodes showed to be activated in the earlier stage, but less activated later in the implantation. Additionally, both WS- and FB-SPCL scaffolds showed to be well integrated in the host, independently of the site of implantation.

Conclusions: The overall data suggests a good integration of the materials in the host, independently of the tissue location. The results showed that the SC implantation induced a slightly higher inflammatory response than the IM implantation with early activation of the lymph nodes. Nonetheless, a normal progress of the reaction was observed for all the conditions.

O22 (EI0306)

THE EFFECT OF THE DIFFERENTIATION STAGE OF AMNIOTIC FLUID STEM CELLS SEEDED ONTO BIODEGRADABLE SCAFFOLDS IN THE REGENERATION OF NON-UNION DEFECTS

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Objectives: Bone tissue engineering strategies mainly require cells with high proliferative and osteogenic potential, and a suitable scaffold to support cellular development towards neobone formation. Amniotic fluid stem cells(AFSCs) have shown high self-renewal capability, and the potential to differentiate along the osteogenic lineage, while SPCL [blend of starch and poly(ε-caprolactone)] fiber mesh scaffolds, developed by melt bonding, have shown promising results for bone applications.Therefore, in this study we have evaluated the functionality of SPCL scaffolds seeded with human AFSCs *in vitro* and *in vivo*. Furthermore, the influence of the differentiation stage of AFSCs on the regeneration of femoral non-union defects was investigated in a nude rat model.

Methods: AFSCs were seeded onto SPCL scaffolds and *in vitro* cultured for different periods of time in osteogenic medium in order to obtain: i) undifferentiated cells, ii) cells committed to the osteogenic phenotype and iii) "osteoblastic-like" cells. After these end-points, cells were assessed and characterized for viability, osteogenic phenotype and matrix formation (ALP,

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SEM, immune fluorescence for collagen I, and calcium quantification assays). Afterwards, SPCL constructs with AFSCs at different stages of differentiation were implanted (4 or 16 weeks) for assessment of bone regeneration by m-CT analysis, and immune-histological characterization (osteocalcin, collagen I and VEGF).
Results: AFSCs proliferated on the SPCL scaffolds, and showed a cellular commitment towards the osteogenic lineage after 2 weeks, with the production of a mineralized ECM after 3 weeks in osteogenic medium (osteoblast-like AFSCs). *In vivo* neoformation of bone was observed in all conditions. Nevertheless, the best bridging between the two sections of the defect was observed in the presence of SPCL scaffolds seeded with osteogenically committed AFSCs after 16 weeks. Blood vessels were also observed in the inner sections of constructs implanted with AFSCs.
Conclusions: Results indicate that SPCL scaffolds combined with AFSCs evidence great potential of for bone regeneration in non-union defects.

Cardiovascular General 2: Devices Interaction – *GENERAL SESSION*

O23 (EI0223)
DEVELOPMENT OF A BLOOD PRESSURE SIMULATOR
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Objectives: The blood pressure simulator is needed for the development of a new method to measure blood pressure (BP) noninvasively over 24 hours. It has to provide a pressure curve similar to the blood pressure in the radial artery. In addition, it has to provide curves with added artefacts as they result from arm movement.
Methods: The blood pressure simulator is driven by a linear motor acting on a piston. The piston is filled with air, which acts as a spring generating a pressure on an enclosed volume. The enclosed volume is filled with the model fluid, which is water containing polystyrene particles. They serve as reflectors for ultrasound waves, since the BP simulator is used for the development of a method using ultrasound to measure blood pressure noninvasively. A water column is connected to the volume generating a hydrostatic pressure p_{hydro}. The model fluid exits the blood pressure simulator and enters a model artery made of thin polyurethane foil embedded in a tissue model made of gelatine.
Results: With this simulator any arbitrary pressure curve $p(t)= p_{hydro}+p'(t)$ can be generated in the artery model. The pressure fluctuation p'(t) is generated by the piston movement x(t) by a linear transfer function $p'(t)=k*x(t)$ with transfer coefficient k. The transfer coefficient depends on the piston area and the spring constant of the air spring.
Conclusions: The blood pressure simulator is able to generate complicated pressure curves as they are found in the radial artery of a moving arm. It is such well suited for the development of a new method to measure blood pressure noninvasively.

O24 (EI0136)
NONINVASIVE BIOLOGICAL PARAMETERS MEASUREMENT IN HEART PROSTHESIS
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Objectives: For optimization, monitoring and partial-automation of mechanical heart support it is necessary to measure appropriate biological signals. The paper presents noninvasive measurement methods of those parameters. Goal of this study was to construct and examine selected biological parameters measurement methods dedicated to pulsatile VAD. This work is a part of the Polish Artificial Heart project.
Methods: The following biophysical quantities measurement methods were investigated: blood flow (ultrasound Doppler velocity profile methods), temporary blood volume in the pump (Helmholtz resonance and rheoimpedance methods), blood pressure (piezoresistive sensors separated from blood through the polyurethane membrane) and epicardial ECG. Investigations were performed on *in-vitro* models, which simulated essential biophysical phenomena. Trial measurements were performed on animals (110kg pig).
Results: Accuracy of Doppler volumetric blood flow measurement was 20%. For the rheoimpedance method, the difference between measured and reference volume (calculated by flow integration) was 0.44mL with standard deviation=3.5mL (Bland-Altman plot). Helmholtz resonance method allowed to measure blood chamber volume with unreliability of 5%. Accuracy about 3mmHg and negligible hysteresis were obtained for blood pressure measurement gauge. QRS detector efficiency (estimated according to EN-60601-1-2-47 regulation) was > 99%.
Conclusions: The performed investigations allowed to select measurement methods appropriate to utilization in final construction of pulsatile VAD. The following methods were chosen: Doppler blood flow, pressure measurement by piezoresistive sensor and QRS detection from epicardial ECG. While developing VAD manufacturing technology the gauges construction limits should be taken into consideration.

O25 (EI0133)
ANALYSIS OF CEREBRAL MICROEMBOLISM DURING CARDIOPULMONARY BYPASS DEPENDENT ON CANNULA POSITIONING: A COMPUTATIONAL STUDY
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Objectives: Cerebral microembolism (CM) is a common problem in cardiopulmonary bypass (CPB) patients, resulting in postoperative neurologic malfunction. While the role of arterial line filters has been thoroughly studied, the possibilities to decrease CM by cannulation techniques have been neglected. In this study, a numerical model is presented to analyze clots behavior in the blood flow dependent on cannula position. To achieve this, the tracking of blood clots was implemented in a particle image velocimetry validated computational fluid dynamics (CFD) model.
Methods: CFD simulations of CPB conditions with different cannula positions were performed in a 3D-model of the cardiovascular system, which was derived from MRI data. Carotid and vertebral arteries were included to represent the cerebral vascular structure. 2000 clots with diameters of 100-500 micron were inserted through the cannula. The path of each clot was tracked to analyze conditions under which clots are washed into the brain.
Results: The behavior of clots was affected by the positioning of the outflow cannula. A cannula tilt towards the cerebral vessels resulted in an increased likelihood for CM. In general, most clots reached the descending aorta and thus the peripheral vessels. Approximately 5% of clots below 200 micron and 2% of clots between 200 and 400 micron arrived at the outlets representing cerebral vessels, predominantly the carotid arteries. Less than 0.5% of larger clots were washed into the brain.
Conclusions: This model provides the possibility to analyze different cannulation methods during CPB in terms of CM, allowing for better understanding of this phenomenon and thus better patient outcome. The first results indicate that clots with smaller diameters are more likely to be washed into the brain. These clots have also a higher probability to escape arterial line filters. Simulations with different cannula designs and clot sizes below 100 micron are currently ongoing.

O26 (EI0123)
EFFECTS OF PULSATILE AND CONTINUOUS MECHANICAL CIRCULATORY SUPPORT ON REGIONAL ORGAN FLOW. EXPERIMENTAL STUDY WITH COLORED MICROSPHERES
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Objective: To measure the regional blood flow in different organs in healthy minipigs with a pulsatile VAD and a continuous centrifugal pump, first in conditions of total support and after in partial support conditions.
Material and Methods: Eight healthy minipigs were used for this study. In four of them a Berlin Heart Excor VAD had been implanted (pulsatile flow) and in the other four a Biomedicus centrifugal pump had been used (continuous flow). In both cases the inflow cannula had been connected to the apex of the left ventricle and the outflow cannula anasthomosed to the ascending aorta. Once the pump was placed, a first (basal) injection of yellow microspheres in the left auricle was performed. Then the pump was started and working parameters adjusted to achieve the maximum pump flow (Total support). These conditions were maintained during 30 minutes; subsequently, a second injection of eosin microspheres was performed. Then the pump flow was reduced to a half of the

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maximum flow (partial support) and maintained during the next 30 minutes, after that, a third injection of violet microspheres was performed. Finally the animal was sacrificed and samples of myocardium, kidneys, lung, liver, bowel and brain were obtained to measure the regional blood flow during the experience.
Results: During total support the flow in kidneys and lung increases to levels around 150% as compared with the basal flow either in pulsatile or in continuous groups. In partial support conditions the blood flow remains around the basal levels in both groups. In myocardium, liver, bowel and brain modifications in regional blood flow are not significant.
Conclusions: Kidneys and lung have a different behavior in terms of regional blood flow during total support conditions as compared with myocardium and other organs studied despite of the flow pattern, pulsatile or continuous

O27 (EI0180)
EFFECT OF INFLOW CANNULA ON ROTARY BLOOD PUMP SPEED PULSING STRATEGY
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Objectives: Timing impeller speed change with respect to the native cardiac cycle to induce pulsatility in Rotary Blood Pumps (RBPs) applications could influence both ventricle volumes and heart unloading, and thus the potential for myocardial recovery. However, the physical properties of inflow cannulas (resistance, inertance, compliance) could also alter the result of these pulsations. The purpose of this study was to determine the appropriate physical properties of cannula to fulfill the desired effect of these pulsations on hemodynamic.
Methods: A mock circulation loop was operated to produce left heart failure conditions. A mixed flow RBP, connecting from ventricle to aorta, was configured to alter rotational speed, with pulse peaks in systole or diastole. Tubings with different length, inner diameter and material were used as the inflow cannula. Pressure at both ends of cannulas, motor power, resulting hemodynamic measurements of ventricular and aortic pressure, VAD outflow, ventricular volumes, and stroke work were recorded.
Results: For pulse peak in systole, pressure drops through inflow cannula were increased from ~2mmHg with 12mm (inner diameter) tubing to ~5mmHg with 3/8" tubing, with a phase delay of desired pulse peak increasing from ~0.028s to ~0.056s. 12mm tubing with 2.33 times more than the original length resulted in further phase delay of pulse peak (~0.069s), but a lower pressure drop (~4mmHg). Silicone 12mm tubing produces a slight increase of pressure drop (~3mmHg), and a rise in phase delay of pulse peak (0.111s), compared to PVC 12mm tubing. The same trend was also found for pulse peak in diastole.
Conclusions: Average hemodynamic pressure remained relatively unchanged for all four cannula tests. 12mm PVC tubing with less than 150mm in length proved the most compatible with pulsatile operation, by providing the lowest pressure drop, smallest phase shift and lowest motor power required for RBP pulsatile strategy.

O28 (EI0009)
A VENOUS NEEDLE DISLODGEMENT METHODOLOGY BASED ON DETECTION OF HEART PULSES IN THE EXTRACORPOREAL CIRCUIT
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Objectives: Accidental venous needle dislodgement (VND) during dialysis is a rare, but serious clinical event. Epidemiological studies, however, indicate that worldwide as many as 200 patients die each year from VND. Modern dialysis machines rely on conventional venous pressure monitoring (CVPM) to detect VND. However, better venous needle monitoring (VNM) is needed because in practice it is difficult to set alarm limits adequately to assure efficacy of VND detection at an acceptable level of false alarms.
Methods: We have developed a new VNM method based on detection of heart pressure pulses passing from the patient via the blood access to pressure sensors in the extracorporeal circuit. An adaptive RLS algorithm is used on the pressure signals to eliminate the relatively large blood pump variations and extract the heart pulses. Disappearance of the heart pulses from the venous pressure indicates VND. Dislodgement is detected with a correlation between extracted venous and arterial heart pulses. High correlation is obtained if both needles are in place, and low correlation indicates dislodgement.
Results: Ten HD treatments and 4 treatments with controlled VND were clinically evaluated. The venous needle was deliberately disconnected from the access during 30 s while the arterial needle was still connected and the blood pump was still running. Both the ability to detect dislodgement and the robustness of the method with respect to false alarms were evaluated. The method was capable of detecting heart pressure pulses in all treatments. All VND events were successfully detected within 20 s after the needle was disconnected. In the majority of the treatments no false alarms were obtained, and in the remaining treatments the number of false alarms was less than with CVPM.
Conclusions: The new VNM method is able to detect VND, and reduces the number of false alarms compared to CVPM.

Artificial Muscle for Internal Organ - *SYMPOSIUM*

K4 (EI0363)
ARTIFICIAL MUSCLE FOR THE ACTUATOR OF THE ARTIFICIAL INTERNAL ORGANS
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Objectives: For the development of the totally implantable artificial internal organs, size and weight are the important issue. Biometal, new shape memory alloy actuator had been used in the development of the various kinds of artificial internal organs like artificial esophagus, artificial sphincter and artificial myocardium.
Methods: Biometal, Ni-Ti SMA, were used for the actuator in the various kinds of artificial internal organs. Crystal structure of the SMA were arranged by the nano technology, so, the durability and contractility of Biometal became useful level. By the use of Biometal, peristalsis movement of artificial esophagus, closing function of the artificial sphincter, and contraction support mechanism of artificial myocardium were embodied. By the use of these systems, animal experiments were performed to confirm the performance, antithrombogenicity and durability by the use of the goats.
Results: As for the results, satisfactory performance of the peristalsis movement of artificial esophagus, artificial sphincter, and artificial myocardium systems were observed. A performace and durability test for artificial sphincter was performed and satisfactory results were observed over 3 months. Performace and durability of artificial myocardium system were evaluated in chronic animal experiments using goats in one month, however, electrical circuit was unfortunatelly broken in some system.
Conclusions: The performance level of Biometal was almost satisfactory in animal experiments, but durability of total system must be discussed. This new artificial internal organ system will represent some good news for the patients in the near future.

O29 (EI0358)
BIOMEDICAL ENGINEERING APPROACH FOR MECHANICAL CIRCULATORY ASSIST USING NOVEL SHAPE MEMORY ALLOY FIBRES
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Objectives: We have been developing artificial myocardial and circulatory assist devices by using sophisticated Ni-Ti anisotropic shape memory alloy fiber (Biometal). Mechanical contraction in the single fibre actuator can be achieved by the application of electric current with simple controllers for the Joule heating, and the long-term durability has been tested for more than one billion cycles. In this paper, we presented the effects of cardiovascular assist devices on hemodynamics and preliminary results on the novel biomedical engineering approach for pediatric circulatory assist based on shape memory alloy fiber technology.
Methods: We developed the myocardial assist devices for the left or right ventricular assistance along with the anatomically sustainable structure and cardiac functions. In vitro and in vivo experiments were performed for the

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examination of the hydrodynamic or hemodynamic functions. Thermodynamic properties in device were also examined during the assistance.
Results: A single 100 micron fiber actuator unit exhibited approximately 0.4kgf as exergy, which was to be used as work. Total weights of the artificial myocardium developed were less than 150g including silicone covers. There were no significant size-related surgical complications around the organs by the installation of the device via left thoracotomy in animal experiments using goats. Pressure and flow increased by 5-10% by mechanical contraction in each device application by using shape memory alloy fibre.
Conclusions: Miniaturized artificial circulatory assist devices were useful for the mechanical circulatory assist. Moreover, the controllability of these devices might be effective for the immediate response on physiological circulatory demand.

O30 (EI0293)
BIOMETAL MUSCLES TO RESTORE CONTRACTILE FUNCTION OF WEAK HEART
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Objectives: Existing VADs are single-ventricle pumps needing anticoagulation. We developed a bi-ventricular external assist device that partially reproduces the physiological muscle function of the heart. This artificial muscle could wrap the heart and improve its contractile force.
Methods: The device has a carbon fiber skeleton fitting a 30-40kg patient's heart, to which a Nitinol based artificial muscle is connected. The artificial muscle wraps both ventricles. The Nitinol fibers are woven on a Kevlar mesh surrounding each ventricle. The fibers are electrically driven with a dedicated control unit developed for this purpose. We assessed hemodynamic performances of this device using a previously described dedicated bench test. Volume ejected and pressure gradient have been measured with afterload ranging from 10 to 50mmHg.
Results: With an afterload of 50mmHg the system has an ejection fraction of 4% on the right side and 5% on the left side. The system is able to generate a systolic ejection of 2.2mL on the right side and 3.25mL on the left side. With an afterload of 25mmHg the results are reduced of about 20%. The activation frequency can reach 80/minute resulting in a total volume displacement of 176mL/minute on the right side and 260mL/minute on the left side.
Conclusions: These preliminary studies confirmed the possibility of improving the ejection fraction of a failing heart using artificial muscle for external cardiac compression avoiding anticoagulation therapy. This device could be helpful in weaning cardio-pulmonary bypass and/or for short-term cardio-circulatory support in pediatric population with cardiac failure.

O31 (EI0271)
URINARY SPHINCTER BASED ON ELECTRONICS AND ARTIFICIAL MUSCLES
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Objectives: The AMS 800™ is the current artificial urinary sphincter (AUS) for incontinence due to intrinsic sphincter deficiency. Despite good clinical results, technical failures inherent to the hydraulic mechanism or urethral ischemic injury contribute to revisions up to 60%. We are developing an electronic AUS, called ARTUS to overcome the rigors of AMS. The objective of this study was to evaluate the technical efficacy and tissue tolerance of the ARTUS system in an animal model.
Methods: The ARTUS is composed by three parts: the contractile unit, a series of rings and an integrated microprocessor. The contractile unit is made of Nitinol fibers. The rings are placed around the urethra to control the flow of urine by squeezing the urethra. They work in a sequential alternative mode and are controlled by a microprocessor. In the first phase a three-rings device was used while in the second phase a two-rings ARTUS was used. The device was implanted in 14 sheep divided in two groups of six and eight animals for study purpose. The first group aimed at bladder leak point pressure (BLPP) measurement and validation of the animal model; the second group aimed at verifying mid-term tissue tolerance by explants at twelve weeks. General animal tolerance was also evaluated.
Results: The ARTUS system implantation was uneventful. When the system was activated, the BLPP was measured at 1.038±0.044 bar (mean±SD). Urethral tissue analysis did not show significant morphological changes. No infection and no sign of discomfort were noted in animals at 12 weeks.
Conclusions: The ARTUS proved to be effective in continence achievement in this study. Histological results support our idea that a sequential alternative mode can avoid urethral atrophy and ischemia. Further technical developments are needed to verify long-term outcome and permit human use.

Functionalized Biomaterials - *SYMPOSIUM*

K5 (EI0444)
FUNCTIONALIZED BIOMATERIALS FOR ORGAN REPLACEMENT AND REGENERATION
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Functionalized biomaterials are under development for medical devices and regenerative medicine to avoid side effects, to allow or inhibit the attachment of specific cells, to deliver drugs and mediators or to guide regeneration processes within the patients' body. This includes the development of new or modified materials with specific properties, e.g. materials offering niches for stem cells and influencing their differentiation or materials delivering mediators on a specific signal from the surrounding tissues or only in a defined cell type. The symposium will focus on some characteristics that can be achieved by designing appropriate biomaterials and on testing methods to prove functionality and safety.

O32 (EI0352)
SPIDER SILK FUNCTIONALIZED WITH HUMAN ANTIMICROBIAL PEPTIDES AS A NOVEL CHIMERIC PROTEIN FOR TISSUE ENGINEERING APPLICATIONS
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Objectives: Genetically engineered fusion proteins offer potential as multifunctional biomaterials for medical use. Chimeric proteins can be formed using recombinant DNA technology by combining nucleotide sequences encoding different peptides or proteins that are otherwise not found together in nature. In the present study, three new fusion proteins were designed, cloned and expressed and assessed for function, by combining the consensus sequence for *Nephila clavipes* dragline spider silk (6mer) with three antimicrobial peptides: human neutrophil defensin 2 (HNP2), human neutrophil defensins 4 (HNP4) and hepcidin. Spider silk was selected for the core polymer due to its potential as a biomaterial to meet the requirements for both mechanical stability and biocompatibility, necessary for bone tissue engineering. The activities of the three different fusion proteins were compared to identify the most useful sequence for biomedical applications.
Methods: The 6mer sequence was fused with the antimicrobial domains HNP2, HNP4 and hepcidin through step-by-step cloning. These proteins were assessed for their antimicrobial activity against Escherichia coli and Staphylococcus aureus through radial diffusion assay. Attenuated-total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was used to assess the secondary structure of the proteins. Cytotoxicity tests were performed to confirm the potential utility of these multifunctional silk proteins in contact with mammalian SaOs-2 cells.
Results: Secondary structure analysis, performed by ATR-FTIR, indicated that silk maintains b-sheet formation capability even after adding the antimicrobial domains, which is good since is responsible for the exceptional mechanical properties of silk. Radial diffusion tests showed that the antimicrobial domains present in 6mer+HNP2, 6mer+HNP4 and 6mer+hepcidin proteins maintained bactericidal activity. Also, these proteins were capable of sustaining the proliferation of SaOs-2 cells.
Conclusions: These new chimeric proteins suggest a new multifunctional approach to generate biomaterials with useful properties, in this case, control of infections due to the addition of the antimicrobial peptides.

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O33 (EI0297)

BIOMIMETIC SURFACE MODIFICATION ON ARTIFICIAL HIP JOINT FOR ELONGATION OF IMPLANTATION LIFE

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Objectives: According to the anatomy of the hip joint, articular cartilage surface is covered with hydrophilic natural polymers. This layer possesses water enough to enhance lubrication of bones. From the point of view of biomaterials design, recently, we have developed an artificial hip joint by using highly hydrophilic and biocompatible phospholipid polymer, poly[2-methacryloyloxyethyl phosphorylcholine) (poly(MPC)], grafted onto the cross-linked polyethylene (PE) surface. We hypothesize that the structure of surface-modified layers might affect the long-term stability, and the poly(MPC) grafted surface might assure the long-term performance of artificial joints.
Methods: Grafting of the poly(MPC) on PE liner was carried out by photoinduced graft polymerization of MPC in aqueous medium. We examined that the effect of structure and performance of poly(MPC) graft layer on wearing of PE liner by hip joint simulator experiments.
Results: The poly(MPC) grafting on the liners increased in hydrophilicity and decreased in friction coefficient, regardless of the cross-linking of the PE liner or the difference in the femoral head materials. During the hip joint simulator experiments ($5x10^6$ cycles of loading), the poly(MPC) grafting layer functioned well. That is, both decreases in gravimetric- and volumetric-wearing (i.e., particle production) were observed, while the femoral head materials did not affect it. The poly(MPC) grafting abrogated the wearing production, confirmed by almost no wearing of the liner surface, independently of the liner cross-linking or the femoral head materials.
Conclusions: We concluded that the poly(MPC) grafting on the PE liner surpasses wearing effectively due to "fluid lubrication mechanism", that is as the same as the natural hip joint. Thus, extending longevity of artificial hip joints is expected. The technology has been applied for the development of artificial hip joint, and clinical evaluation in Japan has been finished.

O34 (EI0115)

BIOMIMETIC MODIFICATION OF POSS-PCU NANOCOMPOSITE USING RESPONSE SURFACE METHODOLOGY

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Objectives: We have developed and patented a novel nanocomposite polymer for biomedical application, the material based on polyhedral oligomeric silsesquioxane (POSS) nanoparticle and poly (carbonate urea) urethane (PCU) polymer. It is widely accepted that biocompatibility depends on the surface properties of the biomaterial, and graft polymerisation is an attractive method to impart a variety of functional groups to it. Since the inert surface of POSS-PCU is not directly suitable for immobilization of such biomolecules, therefore, graft polymerisation of acrylic acid (AA) and collagen over it was carried out (i.e. biomimetic modification). In this study, design of experiment methodology was also used to develop a predictive technique to optimize the operating conditions for grafting well-controlled amounts of AA and collagen.
Methods: Sheets of POSS-PCU were manufactured and the grafting of AA was carried out using a two-step plasma treatment (TSPT). The grafted films were characterised by ATR-FTIR, SEM, and water contact angle (WCA) measurements. The effects of two identified process variables (pretreatment and polymerisation time length); on the grafting density (GD) were investigated and optimized using central composite design in the response surface methodology. To test cell response to the protein gradient surfaces, human umbilical vein endothelial cells (HUVECs) were cultured on the substrates with different amount of bioactive components.
Results: The presence of the AA grafted layers was confirmed by the appearance of a broad peak of the hydroxyl groups in ATR-FTIR spectrum, decreased in WCD, and morphological changes observed by SEM micrographs. It was found that the collagen was immobilized on the POSS-PCU surface with different amounts, and both the attachment and growth of HUVECs were dependent on the GD of it.
Conclusions: These findings suggest that biomimetic modification of POSS-PCU could be an attractive way to improve blood compatibility and patency rate of small-diameter vascular grafts.

O35 (EI0050)

PH VARIATION DURING LAYER-BY-LAYER ASSEMBLIES OF NATURAL AND ARTIFICIAL GLYCOSAMINOGLYCANS TO CONTROL CELL ADHESION

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The study was aimed to develop biomimetic surfaces with possible biospecific cues to obtain control over cell adhesion by exploiting layer-by-layer (LBL) technique. Multilayers of heparin and synthetically made cellulose derivatives with heparinoid properties, prepared at different pH values were used as a tool to guide adhesion of skeletal muscle cells. LBL is a powerful technique based on alternating adsorption of oppositely charged polyelectrolytes (PEL) forms self-assembled nanostructures as multilayers. Application of biogenic PEL allows mimicking properties of the extracellular matrix. Heparin and cellulose sulfate (Cs1.94) as polyanions, while chitosan as polycation were used to prepare multilayers. The cellulose sulfate was applied, as it is cost effective and highly bioactive regarding mitogenic and osteogenic activity. Variation in pH value of PEL was applied to control the physicochemical and biological properties of multilayers. Multilayers were characterized by water contact angle measurements (WCA), surface plasmon resonance (SPR), atomic force microscopy (AFM). Cellular investigations were done using C2C12 cell line. WCA measurements revealed that multilayer growth with alternating change in wettability by adsorption of different PEL is attributed to their functional groups. SPR also showed that mass of adsorbed material was dependent on pH value and type of adsorbed polyanion, which was further confirmed by AFM measurements showing different topographies. A successful control on bioactivity of prepared surfaces was achieved by adjusting the pH value of polyanionic solutions. Cell experiments demonstrated that multilayers assembled at pH 9.0 were more adhesive for C2C12 cells than pH 4.0 layers and possible reasons for such behavior could be given by wetting properties, and charge of outermost layers pH variation leads to different multilayer properties. Multilayers prepared from artificial glycosaminoglycans like natural ones at specific pH conditions can be used as effective tools to obtain bioactive coatings on material surfaces that control cell adhesion.

Cardiovascular General 3: Physiology and Pump Control – *GENERAL SESSION*

O36 (EI0322)

NONINVASIVE EVALUATION OF HEART RATE VARIABILITY IN ROTARY BLOOD PUMP RECIPIENTS USING PUMP DATA ONLY

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Objectives: In order to evaluate autonomic system function the heart rate variability (HRV) has been established as a valuable tool. In this study a method for a continuous monitoring of the heart rate and its variability in rotary blood pump recipients has been developed and evaluated. This method makes use of pump data only.
Methods: Data from 10 rotary blood pump (RBP) recipients was analyzed. An algorithm was developed to estimate HRV parameters and to detect arrhythmic patterns from the pump flow signal. 147 datasets each of five minutes duration were analyzed including data recorded at the ICU, at the normal ward and during catheter-spiroergometry. Average heart rate (HR) and HRV parameters like the standard deviation of the NN intervals (SDNN), the square root of the mean squared difference of successive intervals (RMSSD), and the HRV triangular index (TI), were evaluated. Results were compared to those obtained using the ECG signal.
Results: Medians and interquartile ranges of the HRV parameters calculated from the ECG were: HR: 98.8(89.6-117.1) bpm; SDNN: 17.9(7.1-38.1) ms; RMSSD: 11.9(8-42) ms; TI: 22.4(17-42.2) ms. Bland-Altman analysis showed that the HRV parameters derived from the pump flow were in close accordance with those derived from the ECG (mean±SD of the difference in HR –0.3±0.8 bpm; SSDN 1±4 ms; RMSSD 3±7 ms; TI 6±12 ms). Arrhythmias like atrial

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fibrillation and extrasystolic beats could be detected as well using the proposed method. The accuracy of the HRV estimation method was not affected by changes in hemodynamics, such as during exercise, Valsalva maneuvers or pump speed changes.
Conclusions: Analysis of patient data showed that heart rate and its variability can be robustly detected from the pump flow signal, thus allowing a continuous monitoring of the patient autonomic system derangement and its eventual recovery.

O37 (EI0397)
HEMODYNAMICS OF ATRIAL FIBRILLATION IN ROTARY BLOOD PUMP RECIPIENTS: A SIMULATION STUDY
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Objectives: Atrial fibrillation (AF) is the most common cardiac arrhythmia and is frequently associated with chronic heart failure (CHF). 20-30% of patients classified in NYHA III-IV suffer from AF. In CHF patients with AF the cardiac output (CO) is usually reduced because of the missing "atrial kick" and of a concomitant mitral and tricuspid leakage. Aim of this simulation study was to investigate the hemodynamics of AF during rotary blood pump (RBP) support.
Methods: A numerical model was employed to investigate the differences between normal sinus rhythm (NSR) and AF during RBP support at rest and during physical activity. The model was adapted to reproduce hemodynamics values derived from literature and from a RBP recipient suffering from AF undergoing a catheter-ergometry. Different pump speeds and changes in left ventricular contractility were simulated.
Results: The CO of the RBP recipient suffering from AF is about 10% lower compared to the same heart condition and NSR; left atrial pressures (LAPs) are comparable. During physical activity the CO is also lower but with a much higher LAP (20%). The end systolic left ventricular volume (ESV) is reduced during AF, at higher pump speeds about 30%. In case of a recovering ventricle (improved contractility) and AF the CO increases but with a slight decrease of LAP only.
Conclusions: Since RBP recipients suffering from AF exhibit a lower CO and an elevated LAP during physical activity, a higher pump speed setting may be required compared to patients with NSR. On the other hand a higher unloading of the ventricle during AF results in a lower ESV, increasing the risk of suction. We conclude that the pump speed setting may be more crucial in RBP recipients suffering from AF than in patients with NSR.

O38 (EI0173)
CONTINUOUS FLOW LEFT VENTRICULAR ASSIST DEVICES INDUCE LEFT VENTRICULAR REVERSE REMODELLING
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Objectives: Unloading of the left ventricle with pulsatile left ventricular assist devices (LVADs) induces reverse remodelling shown by a shift towards lower volumes of the passive end-diastolic pressure-volume relationship (passive EDPVR). Today continuous flow left ventricular assist devices are most frequently used for long term mechanical support. We addressed the question whether a continuous flow LVAD also induced a shift towards lower volumes of the passive EDPVR.
Methods: All explants hearts from patients with chronic heart failure without an LVAD (=Group CHF, n=5) and patients bridged to transplant with a continuous flow LVAD (HeartMate II®,Thoractec® corporation) (=Group Assist, n=6) were prepared for measurement. A balloon was inserted inside the left ventricle through the mitral valve. Pressure in the balloon was measured during inflation of the balloon with incremental volumes of saline. Left ventricular capacitance was indexed by the volume at which the pressure reached 30 mmHg (LVV_{30}).
Results: Mean duration of mechanical support in the Group Assist was 366 ± 204 days. The LVV_{30} was significantly lower in patients with an LVAD (Group Assist: 139,4 ± 18,5mL vs. Group CHF: 210 ± 56,2mL; p<0,05). Accordingly NT-proBNP levels at moment of transplantation were lower in the assisted group (Group Assist: 972,7 ± 675,8 ng/mL vs. Group CHF: 5838,7 ± 3546,6 ng/mL; p<0,05).
Conclusions: Reverse remodelling of the left ventricle is marked by a shift towards lower volumes of the passive EDPVR. This shift has been demonstrated in hearts unloaded with pulsatile LVADs. We demonstrated a same shift in hearts unloaded with continuous flow LVADs. Accordingly NT-proBNP levels were lower compared to unassisted chronic heart failure patients.

O39 (EI0244)
***IN VIVO* EVALUATION OF A HYBRID MOCK CIRCULATION LOOP INCLUDING A BAROREFLEX MODEL**
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Objectives: A hybrid mock-circulation-loop (MCL) consisting of electrically controlled hydraulic elements and a software based model of the baroreflex autoregulation mechanism has been developed and constructed to evaluate control algorithms for mechanical circulatory support systems (MCS). The software detects an applied change in central venous pressure (CVP) and automatically adapts the MCL parameters to mimic the physiological response. The interaction of the developed software model and the MCL has been evaluated with in vivo experimental data from animal trials.
Methods: Two rotary blood pumps (MEDOS DP) were integrated in the MCL in a total artificial heart setup, pumping fluid through a systemic and pulmonary circuit. An electro-mechanically variable venous reservoir was added to the previously presented MCL, to accurately change the CVP. Hereby venous vasoconstriction can be mimicked. Upon a change in aortic pressure, the computer model automatically adjusts the MCL parameters peripheral resistance and unstressed volume as it is done by the auto regulatory system. For in vivo validation, the native ventricles of the animal were replaced by the same rotary pumps and blood volume was varied by draining and infusing blood from and to the venous system. The baroreflex autoregulation response was measured through the changing MAoP.
Results: For a change in CVP and a corresponding change in MAoP the model of the hybrid MCL replicates the baroreflex autoregulation mechanism and stabilizes the MAoP. The replicated response in the developed hybrid MCL and the in vivo setting show a similar behaviour. With this new function of the hybrid MCL, a physiologic autoregulatory response to blood loss or a postural change can be simulated.
Conclusions: The hybrid MCL can be used as an in vitro tool to simulate physiological changes in the cardiovascular system. This facilitates the development of new control algorithms for mechanical circulatory support systems.

O40 (EI0100)
VIDEO EVALUATION OF KINEMATICS AND DYNAMICS OF THE BEATING CARDIAC SYNCYTIUM: AN ALTERNATIVE TO THE LANGENDORFF'S METHOD
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Objectives: Many important discoveries in heart physiology have been made possible using the isolated heart method of Langendorff. Nevertheless, this method has some limitations such as the vulnerability of the excised heart to injuries, the preconditioning during instrumentation, the possibility to induce tissue oedema, and a high oxidative stress, leading to the deterioration of the contractile function. To avoid the preceding drawbacks, we have alternatively used beating mouse cardiac syncytia cultured *in vitro* in order to assess possible ergotropic, chronotropic, and inotropic effects of drugs.
Methods: To achieve the preceding aim, we have developed a method based on image processing analysis to evaluate the kinematics and the dynamics of that drug-stimulated beating syncytia starting from the video registration of their contraction movement.
Results: In comparison with the physiological no-drug condition, we have observed progressive positive ergotropic, positive chronotropic, and positive inotropic effects of 10 μM isoproterenol (β-adrenergic agonist) and early positive ergotropic, negative chronotropic, and positive inotropic effects of 10 μM phenylephrine (α-adrenergic agonist), followed by a late phase with negative ergotropic, positive chronotropic, and negative inotropic trends.
Conclusions: The present method permitted a systematic study of *in vitro*

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beating syncytia, producing results coherent with previous works. As a consequence, it could be used in *in vitro* studies of beating cardiac patches, as alternative to the Langendorff's heart in biochemical and pharmacological studies, and, especially, when the Langendorff's technique is inapplicable (e.g., in studies about human cardiac syncytium in physiological and pathological conditions, patient-tailored therapeutics, and syncytium models derived from stem cells with genetic mutations). Furthermore, the method could help, in heart tissue engineering and bioartificial heart researches, to "engineer the heart piece by piece". In particular, the proposed method could be useful in the identification of a suitable cell source and in the development of "smart" biomaterials and novel bioreactors.

O41 (EI0036)
SAFETY AWARE PUMP-CONTROL FOR A ROTARY ECMO BLOOD PUMP
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Objectives: During ECMO, about 12% of all complications are caused by the blood pump or by air in the circuit. In addition, hemolysis, which bases on activation within the blood pump, causes complications during 7% of all ECMO treatments. Since ECMO is still an ultima-ratio therapy we want to improve this situation by introducing a safe, but also automated ECMO setup, which can be run by a closed-loop control.
Methods: We developed a remote steerable closed-loop control for a rotary blood pump. One of the major advantages of rotary blood pumps is a reduced activation of coagulation factors. To increase the safety of the blood pump operation, we introduced a model-based predictor. Thus, we are able to diagnose pump related problems during ECMO like air in the circuit, suction of the cannulae or thrombi in the circuit. Air in the circuit and thrombi can be detected due to a behavior differing from the known pump-characteristics. Suction of the cannulae is detected by blood-flow and pressure gradients, and can be used for fault correction.
Results: Applying closed-loop control, we were able to set a flow within a settling time of 1.08 sec and no overshoot. The introduced model-based supervisor enables us to detect discrete problems within the pump system. Thus, we can significantly enhance the safety of an ECMO treatment.
Conclusions: The proposed pump control enables us to use a rotary blood pump as part of an automated ECMO setup. Due to the embedded safety models, we are able to decrease the risk related to an ECMO treatment. The introduced pump control is a next step closer to an automated ECMO setup.
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Vascular Access in Hemodialysis - *SYMPOSIUM*

K6 (EI0313)
HEMODYNAMICS IN VASCULAR ACCESS FOR HEMODIALYSIS: AN OVERVIEW
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Objectives: Chronic hemodialysis requires easy access to blood flow rates greater than those typically found in the extremities. The high flow, however, may lead to flow disturbances, responsible for the development of intimal hyperplasia, and possibly initiating stenosis formation and/or thrombosis. Therefore, many groups performed experimental as well as computational studies of vascular accesses in an effort to improve access outcome.
Methods: An overview of our methods is presented. First, access models were manufactured using a transparent silicon elastomer to perform hemodynamic studies with a pulsatile flow. Second, access hemodynamics was also studied using Computational Fluid Dynamics.
Results: The vascular access includes generally three regions of interest (ROI) regarding to the hemodynamics: ROI-1 is the arterial anastomosis and its importance for forearm ischemia/steal syndrome and intimal hyperplasia. Different studies were carried out. First, the shape of the arterial anastomosis was changed to investigate the flow pattern in a direct artery-graft connection and with a small diameter segment between artery and graft. And second, AV access-related ischemia was studied, by comparing experimentally different methods of treatment. ROI-2 is the artificial graft itself and the impact of graft length on the flow. And ROI-3 is the venous anastomosis and its trend to the development of intimal hyperplasia. The following venous anastomotic configurations were investigated: conventional end-to-side anastomosis, correctly trimmed Venaflo graft, untrimmed Venaflo graft, open side branch at the vein floor, subclavian loop graft with narrow versus wide inflow, anastomosis with an extreme stenosis at the vein floor, and additional disturbances of flow patterns within the venous anastomosis caused by single-needle hemodialysis. The results of these investigations are presented.
Conclusions: The results here discussed refer to the clinical outcome, and are compared with the results of other selected study groups.

O42 (EI0188)
DEVELOPMENT OF A CAPD CATHETER WITH INFECTION PROOF EXIT-SITE
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Objectives: In Western countries peritoneal dialysis (PD) only plays a minor role compared to hemodialysis. This is partly due to the high risk of exit-site infections, the leading complications with peritoneal dialysis. The infection is caused by a biofilm, originating at the exit-site. Drug-eluting catheters or catheters containing silver particles have not yet solved the problem. Objective of the project is to develop an infection resistant catheter, which uses a mechanical method to permanently prevent the infection.
Methods: The presented catheter is equipped with a protective sleeve, which surrounds the catheter in the skin penetrating area. Subcutaneously the sleeve is folded and the inner end is hermetically sealed to the catheter. It is made of medical grade polyurethane (PUR) and its surface is coated with polyethylene terephthalate (PET) fibers to enable the ingrowth of connective tissue. After the implantation, the protective sleeve is slowly pulled outwards by means of a small traction device at a rate of few millimetres per week. Thus, it can grow out of the skin but still moves fast enough to prevent the down growth of the biofilm.
Results: In a key experiment 6 of the devices were implanted in goats. The catheters remained infection-free over a period of 420 days, while control devices became infected. For the ongoing experiments the catheter setup was improved. So far, 8 catheters have been implanted in 4 goats and show no signs of pocket generation. The experiment is designed to last one year.
Conclusions: The newly developed catheters show good promise for the prevention of infections of the exit-sites of PD-catheters. The principle could also be used for other skin penetrating implants such as power lines of heart assist devices.

O43 (EI0185)
CLINICAL EVALUATION OF A NEW TYPE OF SHORT-TERM HEMODIALYSIS CATHETER WITH A MICRODOMAIN SURFACE
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Objectives: Short-term dual lumen catheters (below DLC) are a useful tool for vascular access in some patients but carry the risk for complications due to thrombosis and infection. For this study, we utilized a DLC coated with a microdomain surface to improve anti-thrombogenicity and compared it with a regular non-coated DLC.
Methods: For the comparison, we utilized the Gambro manufactured GamCath N catheter (below N) and compared it with the GamCath Dolphin catheter (below D). The D catheter has a very smooth surface designed to improve the anti-thrombogenicity of the catheter. We placed the N catheter 41 times and the D catheter 40 times to hemodialysis patients with maximal sterile barrier precautions.
Results: The average age of the N group was 64±17 and 71±14 for the D group. For the majority of patients in both groups, the catheter was placed in the right jugular vein. Complications seen for the two groups were thrombosis in 8 patients (20%) in the N group and 2 patients (5%) in the D group. Infection was observed in 10 patients (24%) in the N group versus 5 patients (12.5%) in the D group. Duration of catheter placement was 15.2±8.3 days versus 22.0±10.1 days showing a statistical significant length of use ($p<0.05$) for the D group.
Conclusions: The new type of DLC with a microdomain surface showed a trend to decrease complications from thrombosis and infection, and showed promising results in terms of prolonging the catheter lifetime.

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O44 (EI0248)
ARTERIOVENOUS FISTULA IN THE ELDERLY: PREOPERATIVE ULTRASONOGRAPHY MAPPING AND CONSTRUCTION BY INTERVENTIONAL NEPHROLOGISTS
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Objectives: The aim of our retrospective study was to evaluate ultrasonography preoperative mapping of vessels before the first attempt of constructing arteriovenous fistula (AVF) in patients aged ≥65 years.
Methods: 204 patients with end-stage renal disease (ESRD), aged 75±6 years, 57% men and 40% diabetics, were included. Arteries and veins of both arms and forearms were preoperatively examined by ultrasonography/Doppler. Inner diameter of veins (under compression) and arteries were measured. Optimal and alternative positions for AV anastomosis were suggested. AVFs and grafts were constructed under local anesthesia, as outpatient procedure, all by interventional nephrologist.
Results. Adequate cephalic vein was present in 54% with a diameter of 4.9±1.1 mm on the right arm and in 59% with a diameter of 4.7±1.2 mm on the left arm. Suitable veins on forearm were recorded in 59% with a diameter of 3.6±0.7 mm on the right, and in 55% with a diameter of 3.7±0.8 mm on the left. Characteristics of arteries: the diameter of cubital artery was 4.5±0.6 mm on the right and 4.5±0.7 on the left. The diameter of the right radial artery was 2.3±0.4 mm, of the left 2.2±0.5 mm. The diameter of the right radial artery was measured less then 2.0 mm in 31%. On the left forearm 37% of such arteries were found. In 76% (156/204) of patients AVF was constructed, with good immediate function in all but 4 patients, in 72% (113/156) on forearm and in 21% (33/156) on arm. Polytetrafluoroethylene (PTFE) grafts were created in 6% (10/156). In 24% (48/204) of patients construction of AVF/PTFE graft was not performed. AVFs were constructed with significant difference in females vs. males (68% vs. 83%), and in nondiabetics vs. diabetics (88% vs. 72%).
Conclusions: Native arteriovenous fistula can be constructed by interventional nephrologists in the majority of elderly patients with ESRD. Diabetics and women were found to be worse candidates for construction of AVF than nondiabetics and men.

O45 (EI0139)
A NOVEL METHOD FOR MEASURING CATHETER LOCK SPILLAGE
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Objectives: Catheters are widely used for blood purification, parenteral nutrition and for the application of drugs. Previous work has focused on the theory and in vitro demonstration of catheter lock spillage caused by the laminar flow profile and by fluid exchange caused by density differences. This work describes a method that potentially allows measurement of catheter lock spillage in vivo without sampling.
Methods: The method is based on the change of the electrical resistance of the catheter when the lock solution is injected. This method was tested in vitro with 46.7% and 4% sodium citrate solution. The catheter tip was placed in a beaker filled with normal saline (46.7% citrate lock) and 5% NaCl (4% citrate lock). A stainless steal rod in the beaker served as one electrode and an Arrow-Johans™ ECG adapter (a luer connector with stainless steel electrode), which was placed on the distal end of the catheter, served as second electrode. Conductivity was measured with a 5V (rms) 310 Hz sinus voltage and a 10 kOhm resistor in series to the catheter. The driving voltage and the voltage drop at the catheter was continuously measured with a program written under LabView (National Instruments).
Results: 47.6% citrate straight catheter: Catheter conductivity dropped following a single exponential curve. Catheter conductivity was reduced by 80% after 10 minutes and by 95% after 90 minutes. Curved catheter: The decay of the conductivity followed an exponential curve but the final value settled at approx. 50% of the difference compared to the straight catheter. 4% citrate (straight and curved): After injection of the lock the conductivity dropped but remained constant. The conductivity dropped further when a 10-fold volume was injected filling the catheter more completely.
Conclusions: These data confirm previous results achieved with methods not applicable for in vivo measurements.

Polymeric Membranes/Blood Interfaces - *SYMPOSIUM*

K7 (EI0301)
HEMOCOMPATIBILITY TESTING OF POLYMERS
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Objectives: Biomaterials are commonly used in various chronic blood contacting applications such as prosthetic blood vessels or stents, etc. Surface properties of polymers (e.g. availability of functional groups, domain structure, electrical charge, hydrophobicity, interfacial adaptability, surface roughness) are assumed to determine the fate of blood proteins and platelets interacting with the materials. Until now, no consistent relationship has been found between hemocompatibility and these parameters. Because thrombogenicity represents the most common cause of graft failure and of stent thrombosis, polymers have to be tested for their thrombotic potential.
Methods: Though the principles of hemocompatibility testing have been established (ISO-10993), they are to be seen as minimum requirements and supplementary tests need to be performed. But, because of the high variability of hemostasis a stringent test procedure and a very rigid stratification of the blood cell donors have to be applied.
Results: Conditions for reproducible measurements will be shown and important pitfalls – that have to be avoided – will be discussed. Basically, there are two options to investigate the hemocompatibility polymers: static and dynamic setups. For static investigations, platelets are dropped onto the material surface and subsequently the adherence, activation, and spreading of the platelets is analyzed. To emulate dynamic testing conditions, closed-loop systems filled with platelet-rich plasma or whole blood are used. Due to the parabolic velocity profile, platelets float next to the tube wall so that interactions between platelets and the material under study are possible during circulation.
Conclusions: Different factors interfere with hemocompatibility testing of polymers. A simple and clear-cut analysis is not yet developed so that a stringent test procedure has to be maintained, and parameters according to the reaction of the platelets, the complement system, the plasmatic coagulation and the contact activation should be accounted for in the assessment of the hemocompatibility of polymers.

O46 (EI0405)
POLYMERIC MEMBRANES WITH TAILORED BARRIER AND SURFACE PROPERTIES
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Membrane technologies have been established in a wide range of industrial processes, and some medical applications are among the most successful examples. However, membranes can offer yet many more distinct advantages when applied as separator or contactor [1]. The majority of synthetic membranes are made from polymers because barrier and surface properties can be varied in wide ranges with the help of established scalable manufacturing processes. Significant efforts have been made to further improve membrane performance by focusing on barrier properties (high selectivity, high flux) or surface properties (antifouling, biocompatibility). Important strategies include the development of novel membrane polymers, which can form ordered self-assembled structures or membrane surface and pore functionalization with controlled functional macromolecular architectures. Membranes with “smart”, i.e., stimuli-responsive barrier or surface properties, can also be created. All these developments had been critically reviewed [2-4]. Important trends, which are most relevant for biomedical engineering, will be discussed. Those will be illustrated with examples from our own research, focusing on ultrafiltration membranes with pH- or temperature-responsive barrier properties, as well as membrane surfaces with minimized fouling, with high protein binding selectivity and capacity, or for the recognition of specific cells. Finally, our contributions to an ongoing multi-partner project for the development of an *in vivo* biosensor (“diagnostic implant”), i.e., the designs of a polymeric hydrogel-based receptor/transducer element and of a blood-compatible polymeric barrier membrane, will be presented.
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O47 (EI0216)

SYNTHESIS AND CHARACTERIZATION OF BI-SOFT SEGMENT POLY-(ESTER URETHANE UREA) (PEUU) MEMBRANES FOR EXTRACORPOREAL BLOOD OXYGENATION DEVICES

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Objectives: Polyurethanes are typically block copolymers that contain an ether/ester soft segment and an aromatic/aliphatic hard segment. The hard-to-soft segment ratio in polyurethanes can be changed by controlling the synthesis parameters, in order to get tailored polyurethanes with improved physical and biocompatible properties. The structural versatility of polyurethanes with two soft segments has been subject of intense investigation in our group for the synthesis of symmetric and asymmetric gas permeable membranes. Having in mind the incorporation of these membranes in blood contacting devices and namely in membrane blood oxygenators, two main goals drove the synthesis of bi-soft segment asymmetric polyurethane membranes: i) The enhancement of O_2 and CO_2 gas permeation rates, and ii) The enhancement of the hemocompatibility.
Methods: Tailoring of bi-soft segment asymmetric poly-(ester urethane urea) (PEUU) membranes with enhanced hemocompatibility through the control of the surface morphology of the top dense layer. Bi-soft segment integrally skinned poly-(ester urethane urea) (PEUU) membranes containing polypropylene oxide (PPO) and polycaprolactone (PCL) as soft segments are synthesized with PCL-diol ranging from 0% – 15% (w/w).
Results: The membrane with 15% (w/w) of PCL-diol shows the smoothest top dense layer with a R_a as low as 1 nm, which is 5 times below the characteristic value of the PEUU membrane with a single soft segment. The PEUU 85 asymmetric membrane displayed minimal platelet deposition and inhibition of extreme stages of platelet activation.
Conclusions: Atomic Force Microscopy characterized sub-micron roughnesses, R_a, of top dense surfaces of the asymmetric membranes as major assets to development of platelet/membrane surface interactions. Here we show that the top dense surfaces of asymmetric PEUU membranes can be tailored with different morphologies when the ratio of the two soft segments PPO/PCL varies. A strong correlation between the top surface roughnesses and platelet deposition is identified.
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O48 (EI0073)

3D-STRUCTURING OF POLY(VINYL ALCOHOL)-BASED PHOTOPOLYMERS

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Objectives: The fabrication of 3D-scaffolds with defined pore geometries, which enable good adhesion of cells, is a challenging topic in the field of regenerative medicine. Photopolymers, which can be structured by means of Additive Manufacturing Technologies, are promising materials for this application. The possibility of structuring these compounds via processes such as microstereolithography (µSLA), Digital Light Processing (DLP) or Two Photon Polymerization (2PP) enables the fabrication of constructs with complex geometries and high resolution mimicking cellular structures of natural materials such as bone.
Methods: Beside the considerable irritancy and sometimes toxicity of acrylate-based monomers, the formation of polyacrylic acid through hydrolytic degradation of the polymer is another undesirable aspect of these materials when applied in the biomedical field. Therefore, photopolymers with different polymerizable groups such as vinylesters, vinylcarbonates and vinylcarbamates, which give water-soluble poly(vinyl alcohol) upon hydrolytic degradation, were evaluated. Several monomers were synthesized to examine the properties of these substance classes with focus on cytotoxicity, photoreactivity, mechanical properties and degradation behavior. 3D-parts made of the new materials were implanted into New Zealand White Rabbits to examine the behavior under physiological conditions.
Results: The biocompatibility of these new substances, measured by their cytotoxicity towards osteoblast-like cells, showed better results than for their (meth)acrylate-based counterparts. The photoreactivity was found to be between that of acrylates and methacrylates, mechanical properties were on the same level and degradation characteristics could be tailored over a broad range. The in vivo studies showed excellent biocompatibility of the materials as well as osteoconductivity due to the layered structure inherent to parts structured with conventional AMTs.
Conclusions: The prepared photopolymers based on poly(vinyl alcohol) show interesting properties for the application in the biomedical field. Under the maintenance of mechanical properties and photoreactivity of conventional photopolymerizable monomers based on (meth)acrylates, cytotoxicity and the degradation behavior could be significantly improved.

O49 (EI0061)

INSIGHTS INTO THE ROLE OF MATERIAL SURFACE TOPOGRAPHY AND WETTABILITY ON CELL-MATERIAL INTERACTIONS

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Objectives: Effects of the nature of biomaterials and surface topography, on protein adsorption, cell attachment, proliferation and morphology are evaluated and reveal important insights in the complexity of cell-material interactions.
Methods: We investigated protein adhesion and C2C12 pre-myoblasts cell culture on biomaterials of various wettability. Materials used were poly(dimethyl siloxane) (PDMS), poly(L-lactic acid) (PLLA), a co-polymer of poly(ethylene oxide) and poly(butylene terephtalate) (PEOT/PBT) and tissue culture polystyrene (TCPS). A micropatterned array of pillars with variable pillar spacing and pillar height was embedded on the biomaterial surfaces through solvent casting of the polymers in solution on specific molds. The morphology of the patterned materials was evaluated by scanning electron microscopy.
Results and Discussion: Our results reveal a clear effect of surface topography, and to a lesser extent of material hydrophobicity, on cell attachment, morphology and proliferation. Generally, surface topography on very hydrophobic materials improves initial C2C12 cell attachment, whereas less hydrophobic and non-patterned materials seem to support higher cell proliferation and spreading. With respect to cell morphology, surface topography seems dominant over material wettability; though, the transition where cells change from growing on top of the patterned pillars to growing on the underlying biomaterial surface appears to be determined by the material wettability.
Conclusions: Surface topography, and to a lesser extent material hydrophobicity, has a clear effect on cell attachment, morphology and proliferation. These findings are important in the design of biomaterials in various applications including medical implants, bio-artificial organs and tissue engineering.

Nano and Micro Technology: driving the future of organ recovery & development - *SYMPOSIUM*

K8 (EI0434)

MICROSCALE TECHNOLOGIES FOR REGENERATION OF FUNCTIONAL TISSUES MODELS *IN VITRO*

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Most tissues in organisms are composed of repeating cellular structures (i.e., functional units), such as the lobule in the liver and kidney, islets in the pancreas. In vivo, the cells in these functional units are imbedded in a three-dimensional (3D) microenvironment composed of extracellular matrix (ECM) and neighboring cells with defined spatial distribution. Tissue engineering approaches therefore attempt to recreate the native 3D architecture in vitro. Recently, the convergence of nano and microscale technologies and hydrogels has resulted in the emergence of bottom-up methods where cell-laden microgels can be used as building blocks for tissue engineering and regenerative medicine. Although various microgel fabrication and assembly methods have been developed based on modifying interfaces and using microfluidics, so far, two main challenges remain: (1) to fabricate microgels composed of multiple cell

types spatially confined in 3D as functional units, and (2) to assemble microgels into large complex 3D constructs rapidly in an efficient way. We also developed a simple, non-invasive acoustic assembler for cell-encapsulating microgels with maintained cell viability. The microgels were assembled via acoustic field in seconds in a non-invasive manner. Besides, we developed novel cell printing technologies where microgel fabrication and assembly are integrated into one system. With cell printing, we have successfully regenerated muscle tissues, created in vitro cancer co-culture models, and engineered controlled niches for embryoid stem cells. These methods that we present would enable a better biologically relevant in vitro platform to investigate cell–cell interactions in a 3D microenvironment, holding great potential in various areas, spanning tissue engineering, regenerative medicine, pharmacological studies and high throughput applications.

O50 (EI0415)

ENHANCED IN VITRO AND IN VIVO OSTEOGENESIS OF RAT BONE MARROW STEM CELLS THROUGH INTRACELLULAR DELIVERY OF DEXAMETHASONE WITH GELATIN MICELLE

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Objectives: Dexamethasone is known to promote osteogenic differentiation of stem cells. The objective of this work is to evaluate the effect of dexamethasone-loaded gelatin micelles and the consequent intracellular delivery on MC3T3-E1 (pre-osteoblastic cell line) and rat bone marrow stem cells (rBMSCs) in vitro and in vivo osteogenic differentiation.
Methods: The internalization efficiency of the micelles was assessed by flow cytometric and confocal laser microscopic measurements after their in vitro culturing with the MC3T3-E1 and rBMSCs. Fluorescent-labeled micelles were added into the culture medium and the cell response was evaluated at different time points. The in vitro release of dexamethasone was also evaluated. The cells pre-incubated with the dexamethasone-loaded micelles, were then seeded in 3D gelatin hydrogels to evaluate the in vitro osteogenic differentiation. The hydrogels with cells were then implanted into a rat critical size bone defect and the bone formation was assessed by x-ray, micro computer tomography and histological analysis.
Results: The internalization efficiency of the gelatin micelles by MC3T3-E1 and rBMSCs was higher than 90% for all the formulations. When cultured in vitro, the dexamethasone-loaded gelatin micelles enhanced the expression level of alkaline phosphatase and mineralization, as confirmed by higher calcium content and stronger alizarin red staining. When seeded into the 3D gelatin hydrogels, both types of cells pre-internalizing the micelles showed enhanced osteogenic differentiation compared with the non-treated cells. The rBMSCs pre-internalizing dexamethasone-loaded micelles and seeded into the 3D hydrogels showed enhanced in vivo new bone formation.
Conclusions: The internalization of dexamethasone-loaded gelatin micelles by MC3T3-E1 and rBMSCs and the consequent intracellular delivery promoted the in vitro osteogenic differentiation and in vivo new bone formation. The present data suggest that the intracellular release of dexamethasone is a promising strategy of bone tissue engineering to improve the efficacy of osteogenic differentiation.

O51 (EI0134)

INVESTIGATION OF A COMPUTATIONAL ANALYSIS FOR GASEOUS TRANSFERS BETWEEN THE BLOOD LAYER AND THE GAS LAYER IN A SEGMENT OF A HOLLOW FIBER BUNDLE OF AN OXYGENATOR

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Objectives: We tried to estimate the gas transfer behaviors in a hollow fiber bundle of an oxygenator by a newly developed computational analysis method and compared its results with actually measured gas transfer performance.
Methods: An invented numerical analysis method combines original programs that calculate membrane transfer source and blood-gas reaction source of both oxygen and carbon dioxide with commercialized computational fluid analysis software that can calculate mass transfer and fluid dynamics. The object oxygenator has a rectangular bundle and consists of parallel and staggered arranged hollow fibers. Lengths of a segment model are 30 mm in the blood flow direction (full length of the bundle) and 4 mm in the gas flow direction (sufficient length for comparing with actual data). Velocity conditions were set up with assuming 1, 3 and 5 L/min of the blood flow rate and 1 of the ratio of gas to blood flow rate. Conditions of inflow blood were set at the AAMI values in both computational analysis and in vitro experiment (PO_2: 37 mmHg, PCO_2: 45 mmHg and Hb: 12 g/dL). PO_2 and PCO_2 of ventilated gas were set at 713 mmHg and 0 mmHg.
Results: The computational analysis demonstrated 72.5, 170.4, and 232.0 mL/min O_2 transfer rates and 66.1, 156.0, and 205.3 mL/min CO_2 transfer rates at 1, 3, 5 L/min blood and gas flows, respectively. In in vitro experiment, O_2 transfer rates were 55.4, 153.1, and 229.4 mL/min and CO_2 transfer rates were 99.5, 117.5, and 151.9 mL/min at 1, 3, 5 L/min blood and gas flows, respectively.
Conclusions: We conclude that newly developed computational analysis is a promising method for estimating gas transfer performance in an oxygenator, although further verification is necessary.

O52 (EI0346)

FUNCTIONALIZED FIBRES FOR SUSTAINED DELIVERY OF BIOACTIVE SUBSTANCES VIA EMULSION ELECTROSPINNING

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Objectives: Functionalization of electrospun fibres is one of the major challenges for their use as scaffolds in tissue engineering. Most biodegradable polymers require organic solvents for electrospinning, which typically denature the bioactive molecules. Hence, we describe here a modification to the traditional process, called emulsion electrospinning, to circumvent this problem.
Methods: Poly(caprolactone) (PCL) was dissolved in chloroform at different concentrations, with Span 85™ added as an emulsifier. The dispersed phase consisted of albumin and alkaline phosphatase (AP) dissolved in PBS. Alternatively, aqueous Eosin-Y solution was used for fluorescence microscopy. Stability of emulsions over time was studied using spectrophotometry. Fibre morphology was observed using scanning electron microscopy (SEM). For release kinetic studies, protein concentration was measured using Bradford assay. AP activity was measured using p-nitrophenyl phosphate as the substrate.
Results: The stability of emulsions showed a distinct dependence on the polymer concentration in the solution with an optimum range between 15% - 20w/v PCL. SEM observations of fibre morphology also confirmed this range to be the most suitable for electrospinning. The proteins were released in a sustained manner from the fibres. AP released from the fibres retained up to 70% specific activity at day 3, indicating that the proteins did not denature during the process.
Conclusions: A method was developed to produce functionalized polymer fibers using emulsion electrospinning. Best results were obtained with emulsions having PCL concentrations between 15% and 20%w/v. Using alkaline phosphatase, it was shown that the released enzyme was active and did not denature extensively during the process. Release of proteins over a longer period, as well as experiments to determine the cytocompatibility are currently in progress.
Acknowledgements: *This work is supported by funding from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) for the Cluster of Excellence REBIRTH (From Regenerative Biology to Reconstructive Therapy).*

O53 (EI0002)

DEPOSITION TRANSFECTION TECHNOLOGY USING A HOMOPOLYMER WITH BOTH THERMORESPONSIVE AND CATIONIC CHARACTERS FOR CARDIAC GENE THERAPY

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Objectives: Effective cardiac gene therapy requires safe and effective gene delivery into the myocardium. Previously, we attempted a deposition transfection technology for a novel non-viral gene transfection method, in which DNA complexes were kept in contact with their deposition surface using block copolymer with a cationic poly(*N,N*-dimethylaminopropyl acrylamide) chain and a thermoresponsive poly(*N*-isopropylacrylamide) chain. In this study, long poly(*N,N*-dimethylaminoethylmethacrylate) homopolymer with both

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thermoresponsive and cationic characters was developed as the material for deposition transfection method in cardiac gene therapy.
Methods and Results: The polymer with molecular weight of 250 kDa was synthesized by photopolymerization. Complex formation between the polymer and plasmid DNA occurred immediately upon simple mixing in an aqueous medium; polyplexes ca. 40 nm in size were formed. Because the lower critical solution temperature of the polyplexes was approximately 32°C, they could deposit on the substrate by precipitation from an aqueous solution upon warming at 37°C, which was confirmed by x-ray photoelectron spectroscopy (XPS) for surface atomic analysis and water contact angle measurement. When Hela cells were cultured on the polyplexes-deposited substrate in a culture medium, the luciferase activity obtained was higher than that observed on a DNA-coated substrate and by conventional solution transfection using the polyplexes. By FACS measurement about 20% of cells were transfected for over 2 weeks with permissible cellular cytotoxicity. In vivo transfection activity of the system was investigated in rat. The polyplexes were injected directly into myocardium of rats and a higher level of Lac Z gene expression was obtained as well.
Conclusions: A highly efficient novel non-viral vector based on deposition transfection technology using the thermoresponsive and cationic polymer was developed for the myocardium of rats.

Roadbumps for tissue-engineering artificial organs – *SYMPOSIUM*

K9 (EI0442)
CURRENT AND FUTURE STRATEGIES TO ADDRESS MANUFACTURING CHALLENGES IN TISSUE AND ORGAN ENGINEERING
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Despite the compelling clinical need to regenerate damaged tissues/organs, impressive advances in the field of tissue and organ engineering have yet to result in viable engineered tissue products with widespread therapeutic adoption. The main challenges to be overcome have been identified in the yet not convincing benefit of the proposed therapies, combined with their high costs. Following the exemplifying paradigm of bone and cartilage regeneration, the lecture will highlight the bottlenecks of typical manufacturing strategies and will propose alternative bioreactor-based approaches for the manufacturing of 3D cellular grafts. The perspective will address issues related to quality standardization, process control and regulatory compliance in manufacturing cell-based products and highlight the need not only to automate, but also to streamline and simplify typical production processes. Examples will be given on the attractive paradigm to expand mesenchymal stem/progenitor cells from adult individuals directly in a "3D niche" environment, thereby maintaining a larger post-expansion differentiation capacity and bypassing the complex and costly serial cell passaging in monolayers. Finally, as a next generation paradigm, the lecture will propose and exemplify the concept of engineering regenerative strategies following principles of developmental biology, using the own body as the in vivo bioreactor.

K10 (EI0441)
PROBLEMS AND POTENTIAL SOLUTIONS TO GROWTH FACTOR APPLICATION IN TISSUE AND ORGAN ENGINEERING
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Most of the cells used for tissue and organ engineering (TE) need growth factors (GF) for survival proliferation and differentiation. While in vitro some GF can be part of the cell culture/perfusion medium, there is the necessity for local retention/application of GFs in vivo. Since those GF are typically needed over a longer period, a sustained (delayed) release or a local production is the goal. Therefore examples will be given for delayed release system by encapsulating and/or binding of GF to TE scaffolds. On the other hand local production will be discussed either from accessory cells (e.g. MSC) or from ex vivo transfected cells or from endogenous or applied cells, which are transfected in vivo. Different transfection systems will be shown, with special emphasis on gene activated matrices and on non-viral transfection systems. The latter technology holds great promise especially for using the body as its own bioreactor.

Artificial Liver - *GENERAL SESSION*

O54 (EI0420)
IMIDAZOLE CYCLODEXTRIN (IMCD), TOTALLY SYNTHETIC SUPRAMOLECULAR COMPLEX AS AN ANTIDOTE FOR CYANIDE POISONING
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Objectives: Cyanide (CN^-) poisoning has been recognized as one of the causes of premature deaths on building fire sites. Whereas sodium thiosulfate has been used to oxidize hemoglobin to methemoglobin to chelate CN^- ion, it is risky since it reduces O_2 carrying capacity of blood even less. We have synthesized imidazole cyclodextrin (ImCD), a totally synthetic supramolecular complex, which has extremely high affinity to CN^- ion. The purpose of this study is to evaluate the effect of ImCD for acute CN^- poisoning in cell culture *in vitro*, and the survival rate in mice as an antidote for CN^- *in vivo*.
Methods: *In Vitro*: Murine 3T3 fibroblasts were incubated for 4 hours with 20% fetal bovine serum (FBS 100µL, FBS group), 20%FBS and KCN 5mM (KCN group), or various doses of ImCD (0.6 5.0 mM) and KCN 5mM (ImCD group). Viability of the fibroblasts was determined by the activity of cytochrome-c-oxidase using MTT assay. *In Vivo*: Two-day survival rate was defined in BALB/c mice, pretreated with ImCD (0.023 mM/kg equi-molar to KCN) or saline, receiving KCN per oral at 0.023mM/kg or LD_{100} dose.
Results: The viability of incubated 3T3 fibroblasts, OD value in MTT assay, was significantly lower in the KCN group (0.027±0.014) than in ImCD (5.0 mM) group (0.379±0.010, $P<0.001$), which was not significantly different from the value in the FBS group (0.380±0.014, $P=0.892$). While dose-dependent antagonistic effect of ImCD was demonstrated *in vitro*, mortality rate of the ImCD-treated mice (33%) was lower than that of saline-treated control mice *in vivo* (100%).
Conclusions: The results suggest that ImCD has a potent antagonistic effect against CN^- cytotoxicity with a dose-dependent manner *in vitro*, and that ImCD has a possibility to reduce the mortality in CN^- poisoning *in vivo*.

O55 (EI0390)
CHARACTERIZATION OF DIFFERENT ACTIVATED CARBON BEADS FOR BLOOD PURIFICATION: ADSORPTION PROPERTIES AND BIOCOMPATIBILITY ANALYSIS
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Objectives: A critical issue of the clinical syndrome in liver failure is the accumulation in the bloodstream of toxins not cleared by the failing liver. The removal of hydrophobic, albumin-bound substances can be extracorporeally achieved by artificial adsorption devices. Here, we report a study on the adsorptive capacity of CO_2 activated carbon spheres (average diameter 300µm) with increasing activation levels (up to 86%). Additional analyses on the biocompatibility profile are presented.
Methods: The adsorption properties of the carbon-based adsorbents were investigated in batch experiments through incubation with spiked human plasma under static conditions at defined time points. The adsorption of hydrophobic compounds accumulating in liver failure (i.e. bilirubin, cholic acid, phenol and tryptophan) as well as pro-inflammatory cytokines (TNF-α, IL-6) was assessed. Furthermore, both the activation of coagulation (monitored by intrinsic and extrinsic coagulation cascades) and the adsorption of individual factors were assessed.
Results: The activation process results in the burning of amorphous carbons, leading to an increase in the specific surface area, the development of microporosity and a substantial shrinkage of carbon particles. Cytokines adsorption rose with increasing burn-off levels (39.2 pg TNF-α/mg adsorbent 86% activation and 6.3 pg TNF-α/mg adsorbent with 0% activation, after 60 min incubation). High adsorption efficiency of all the tested carbon beads for the removal of phenol, tryptophan and cholic acid was observed, however, the adsorption capacity for strongly albumin bound bilirubin did not increase significantly with progressive activation. No hemolysis could be detected for extracts of any of the tested adsorbents, and no abnormal time in the extrinsic and intrinsic coagulation pathways was recorded after incubation.
Conclusions: Increasing burn-off levels enhanced the adsorptive performance

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of carbon particles, predominantly with respect of smaller adsorbates (IL-6, tryptophan, phenol, cholic acid).

O56 (EI0202)
HISTOMORPHOMETRIC ANALYSIS IN THE PROGRESSION OF HEPATIC STEATOSIS
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Objectives: The objective of this study is to create a method which can be easily used to accurately determine the degree of steatosis through an analysis of histomorphometric images, in order to provide a histological objective response.
Methods: For this study 200 patients were randomly enrolled, including: 30 patients with normal histological examination performed for suspected disease, 170 patients with positive histology for different grades of steatosis. Two hundred images were analyzed for a total of 2275 sinusoids. The portions of each image occupied by sinusoids were outlined and the number of pixel of each sinusoid was calculated. The total area occupied by sinusoids within each specimen was then computed and reported. We have analyzed the form of sinusoids approaching this with an ellipse and then make the relationship between the two axes with the aim of measuring the hydraulic resistance.
Results: We found a non-linear progression in morphological and functional parameters between the different degrees of steatosis: hydraulic resistance, number of sinusoids [mm^2]; percentage of sinusoids in a frozen section, area of the sinusoids and form/area ratio of the sinusoids.
Conclusions: This correlation between geometric analysis of images of individual histological changes of sinusoids could be predictive of the clinical evolution of disease. These results are not statistically significant, but suggestive to apply this methodology in a larger number of patients.

O57 (EI0200)
DELAYED GRAFT FUNCTION IN LIVER TRANSPLANT. ROLE OF MOLECULAR ADSORBENT RECIRCULATING SYSTEM
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Objectives: The aim of this study is to highlight the capacity of albumin dialysis MARS to improve the clinical status in liver transplant patients affected by delayed graft function (DGF).
Methods: From 1999, twenty-seven patients affected by DGF were treated with MARS. Eighteen patients with similar MELD[20-25] score were enrolled in the study (Group A). The reference population of eighteen DGF (Group B) that showed similar MELD score was treated with Standard Medical Therapy (SMT). The number of MARS applications was 9±2.2; the length of applications was 8h. Serum values with relation to inflammation, excretory and detoxification function, synthesis capacity, renal function and hemodynamic parameters were measured using standard laboratory procedures before and at the end of MARS treatments. The patients were valued for 30 days from inclusion with a survival follow-up at six months.
Results: In patients treated with MARS therapy, all the variables detected in our analysis showed an evident improvement. The survival at 30 days and at three months was 100% and 95%, respectively. Within three months, one patient underwent liver transplant (LT). In Group B we obtained a survival at 30 days, and at three months 88% and 61%, respectively. Five patients underwent LT.
Conclusions: MARS therapy was able to improve clinical status and survival in patients with DGF. Moreover the use of MARS avoided the re-transplant of 89% patients. Further studies including larger numbers would be useful in confirming our findings.

O58 (EI0088)
TEST EAA FOR ENDOTOXIN ACTIVITY IN ACUTE ON CHRONIC LIVER FAILURE
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Objectives: The aim of our study is the early detection of endotoxins in patients with acute liver failure on chronic liver disease by the test of EAA and treated with Polymyxin-B hemoperfusion based (PMX-DHP) and Molecular Adsorbent Recirculating System (MARS), with follow-up at 30 days.
Methods: From 2008 to 2010, ten AoCLF patients with SIRS in association to suspect of infection and EAA-positive test (> 0.60) were included in the study. These patients, on a waiting list for liver transplant (LT), showed similar MELD score [range 19-25] and Encephalopathy Grade ≤2. Five patients were treated to remove endotoxins with PMX-DHP and MARS treatment for liver failure (Group A), while the other five patients received MARS treatment only (Group B).
Results: In Group A, two treatments PMX-DHP were performed on 4 patients (average EA=0.66 [from 0.61 to 0.70]), three treatments for a patient (EA=0.92) and all five patients underwent an average of four MARS treatments (range 3-5). At the end of the therapy, the median level of EA was 0.42 (range from 0.37 to 0.48). Measurements of lactates, IL-6, TNF-α were significantly improved in patients treated with these extracorporeal therapies. At 30 days from the observation, all five patients treated with MARS and PMX-DHP are alive. In Group B, a mean of 7.5 MARS treatments were performed. We observed an improvement in hemodynamic and liver functions with reduced levels of pro-inflammatory cytokines and lactates in 4 patients. One patient showed no improvement in clinical status with the development of sepsis and subsequent MOF after 24 days.
Conclusions: The possibility of an early diagnosis with the EAA in AoCLF patients could prevent the progression of sepsis cascade. The use of PMX-DHP and MARS in these patients, could lead to a resolution of the clinical status in a short time.

O59 (EI0047)
DEVELOPMENT OF MATERIAL SYSTEMS FOR ENGINEERING OF BIOHYBRID LIVER
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Objectives: Biohybrid liver represents a promising therapeutic system to sustain life of patients that suffer from acute hepatic failure but also in chronic liver diseases, in order to bridge time to find a suitable transplant. One issue in biohybrid liver systems that has been rarely addressed is the development of membrane materials for the bioreactor that promote both hepatocyte attachment and inter-cellular contacts to maintain survival and function of cells. Here, specific polymers have been synthesized and also the porosity of membranes has been adjusted to achieve this ambitious goal.
Methods: Membranes were made from polyacrylonitrile or copolymers from acrylonitrile with N-vinylpyrrolidone, sodium methallylsulfonate or aminoethylmethacrylate by phase inversion. Porosity of membranes was in ultrafiltration range. One of the copolymers was also used to obtain a series of membrane materials with pore sizes ranging from 6 to 12 nm. Surface properties of membranes were studied with atomic force microscopy, water contact angles measurements and other methods. Biological studies were carried out in most cases with C3A hepatoblastoma cells but also with primary rat hepatocytes.
Results: Copolymerization of acrylonitrile with the co-momoners resulted in membranes differing in wetting properties ranging from hydrophilic to moderate wettable materials. C3A hepatoblastoma cells grew particularly well on moderate wettable membranes having a more spread phenotype, while more hydrophilic membranes promoted intense cell-cell contacts with reduced growth but improved function in terms of cytochrome P450 activity. A change of porosity of hydrophilic membranes had also effects on growth and functional activity and could be used as additional tool to modulate hepatocyte behavior in the desired direction.
Conclusions: Although promising effects of chemical and topographical properties of these novel polymer membranes on hepatocytes were shown and also protected by a variety of patents, none of the membranes has been used in commercial bioreactors so far, which will be discussed, too.

Tissue Engineering Approaches - *SYMPOSIUM*

K11 (EI0437)
THERMO-SENSITIVE HYDROGEL AS CELL CARRIER FOR NUCLEUS PULPOSUS TISSUE ENGINEERING
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Intervertebral disc degeneration usually starts at the nucleus pulposus. In the past decades, several techniques and prosthetics (artificial disc) have been

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developed to regenerate or replace the nucleus pulposus. However, these kind of pre-formed devices have to remove the nucleus pulposus and then replace an artificial one to relieve the symptom of intervertebral disc degeneration. Recently, cell-based tissue engineering has provided a rational approach to regenerate active nucleus pulposus cells (NP cells) to restore intervertebral disc architecture and function. However, the source of autologous nucleus pulposus cells are limited and their functional state does not favor regeneration. Besides, nucleus pulposus cells grown in monolayer may result in fibroblast-like transformation. Thus, the 3D hydrogel co-culture system may be an alternative method to provide an adequate environment for nucleus pulposus cells proliferation, extracellular matrix production, cytokines secretion. In this study, we demonstrated that cell proliferation, total DNA and sulfated glycosaminoglycans synthesis of nucleus pulposus cells were significantly increased in the 3D hydrogel co-culture system. Furthermore, the extracellular matrix related gene expression and anabolism-related gene expression in 3D hydrogel co-culture system were significantly higher than other culture condition (such as monolayer culture or cultured in 3-D hydrogel without mesenchymal stem cells regulation).The gene expression of TIMP-1 and MMP-3 decreased in 3D hydrogel with mesenchymal stem cells co-culture system. This study suggests that the thermo-sensitive hyrogel could be an adequate material for nucleus pulposus cells proliferation and extracellular matrix production. Moreover, mesenchymal stem cells could regulate the isolated nucleus pulposus cells back to normal state through paracrine communications in the developed 3-D co-culture system.

O60 (EI0417)

THE POTENTIAL OF OSTEOGENIC CELL SHEETS CO-CULTURED WITH ENDOTHELIAL CELLS FOR BONE TISSUE ENGINEERING

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Objectives: Current strategies in the field of bone Tissue Engineering are limited by the use of scaffolds that present drawbacks such as cell necrosis at their bulk related to deficient vascularization after implantation. Cell sheet (CS) engineering has been proposed as a scaffold-free alternative for the regeneration of several tissues. This work proposes the use of this technology for bone regeneration by combining osteogenic CSs and endothelial cells.
Methods: Osteogenic CSs were fabricated by culturing male rat bone marrow cells (rBMSCs) in thermo-responsive culture dishes in osteogenic medium. Human umbilical vein endothelial cells (HUVECs) were seeded on the rBMSCs to create co-cultured CSs. The osteogenic CSs were recovered by lowering the temperature and then stacked on top of either a co-cultured or a similar osteogenic CS, and transplanted to female nude mice. Implants were recovered after 7 days and characterized by hematoxylin & eosin (H&E) and alizarin red (AR) stainings, immunohistochemistry for osterix, osteopontin, SRY (to identify transplanted male rat cells) and CD31, and calcium quantification.
Results and Discussion: H&E and AR stainings showed mineralized tissue formation in the implants both with and without HUVECs. Osterix and SRY immunostaining demonstrated the presence of host and donor osteogenic cells at the mineralization site showing recruitment of host osteogenic cells. HUVECs contribution to neo-vascularization was confirmed by identifying human CD31 cells in blood vessels. Furthermore, calcium quantification results showed a higher degree of mineralized tissue after the transplantation of the constructs with HUVECs.
Conclusions: This work confirmed the potential of transplanted osteogenic cell sheets for bone regeneration as well as the advantage of promoting cross-talk between osteogenic and endothelial cells for improved new tissue formation. The proposed approach avoids the constraints of scaffold use while successfully addressing the important issue of implant vascularization.

O61 (EI0330)

ENABLING TECHNOLOGIES FOR ORGAN PRINTING: TOWARD ORGAN BIOFABRICATION LINE

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Objectives: Organ printing is an emerging solid scaffold-free biofabrication technology or layer-by-layer additive bioprinting of functional 3D human tissue and organ constructs from self-assembling tissue spheroids. Bioprinter is a key tool for organ printing. It is becoming increasingly obvious that in order to bioprint human and organ constructs it is necessary to develop series of integrated automated robotic tools or an organ biofabrication line.
Methods: The scalable technique for tissue spheroid biofabrication employs micromolded recessed template in non-adhesive agarose hydrogel, wherein the cell suspension automatically loaded into the template self-assembles into tissue spheroids due to gravitational forces. Robotic bioprinter for the precise dispensation of tissue spheroids include three essential elements: X-Y-Z axis robotic precision position system, three automated biomaterial dispensers (two aseptic valve sprayers and one automated tissue spheroids dispenser) and computer-based software enabled operational control. The first two dispensers spray sequentially fibrinogen and thrombin and enable instant biofabrication of thin layers of fibrin hydrogel, whereas another robotic dispenser punches tissue spheroids into sequentially sprayed layer of fibrin hydrogel.
Results: It has been demonstrated that the use of micromolded recession in non-adhesive hydrogel, combined with automated cell seeding, is a reliable method for robotic fabrication of uniform size tissue spheroids at large scale. It has been also shown that the combination of hydrogel sprayers and tissue spheroids puncher enables to implement additive biofabrication of 3D tissue construct. The novel irrigation dripping tripled perfusion bioreactor with removable porous removable minitubes has been designed. Mathematical modeling and computer simulation demonstrated that proposed irrigation dripping circuit system will allow maintain viability of printed tissue constructs until the "build in" intra-organ branched vascular system will mature enough for initiation intravascular perfusion.
Conclusions: Thus, presented data strongly indicate that design and development of a fully integrated organ biofabrication line is a challenging but achievable goal.

O62 (EI0395)

MICROFLUIDIC ENCAPSULATION OF CELLS INTO SELF-ASSEMBLING XANTHAN-PHOSPOLIPID AMPHIPHILE FOR CELL THERAPY

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Objectives: We have investigated the synthesis of an amphiphilic polysaccharide, in which a phospholipid is attached to an anionic polysaccharide chain (xanthan gum), and its ability to self-assemble into capsular structures. Moreover, this work aimed to apply a microfluidic platform which can overcome inconveniences related with the heterogeneity of microcapsules produced by conventional systems. The properties and performance of the microcapsules were studied as well the ability of these self-assembled matrices to support the viability, function, and proliferation of encapsulated cells.
Methods: Xanthan gum was conjugated with 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) using carbodiimide chemistry to activate carboxylic groups of xanthan and coupling to amine groups of DOPE. The polysaccharide-lipid amphiphile was characterized by physico-chemical methods, such as ^{1}H Nuclear Magnetic Resonance, Fourier Transform Infra Red spectroscopy, X-Ray Diffraction, Circular Dichroism and Scanning Electron Microscopy. A microfluidic device was used to fabricate microcapsules with controlled size and shape. ATDC5 cells (a murine chondrocytic cell line) were encapsulated within the microcapsules and their metabolic activity and viability were investigated.
Results: The self-assembly of the amphiphilic polysaccharide in physiological ionic strength and pH resulted in the formation of stable hollow capsular structures. Using microfluidics, stable and homogenous microcapsules of xanthan-DOPE with average size around 300 μm were fabricated. ATDC5 cells were encapsulated within the capsules and remained viable and evidencing an increased cellular metabolic activity over 21 days of *in vitro* culture.
Conclusions: By combining self-assembly of xanthan-DOPE and microfluidic microencaspsulation we were able to fabricate microcapsules that provided an adequate environment for cells to survive and proliferate.

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O63 (EI0245)

COMPARISON OF 3D STATIC AND DYNAMIC CULTURES OF HEPATOCYTES ON POROUS PDLLA

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Objectives: A hepatic bio-hybrid construct is a device that marries hepatocytes to a scaffold able to guide cell migration, assembling and functionality. The construct should be able to mimic a liver *in vitro* as much as possible. Due to the difficulties in maintaining viability and hepatic functions over the time, culture conditions can deeply affect the construct growth. A dynamic cell culture system (i.e. a perfusion bioreactor) can be the necessary alternative to a static culture. The objective of this work was the comparison of long living and functional hepatocytes cell seeded PDLLA constructs cultured in static and dynamic conditions.
Methods: Poly(D,L-lactic acid) (PDLLA) porous scaffolds were prepared by salt-leaching method. *In vitro* tests were performed on scaffolds seeded with mouse ANL12 hepatocytes cultured up to 14 days in static and dynamic conditions, by using a perfusion home-made bioreactor. Cell adhesion, proliferation and migration were characterized (Alamar Blue, SEM observation and Confocal Microscopy). Their functionality was evaluated by RT-PCR (Albumin, Plasminogen, EGF, TNF-alpha, Fibronectin).
Results: Dynamic cell cultures showed better behavior with respect of the static conditions, both in terms of overall gene expression and 3D distribution. PDLLA scaffolds showed to be a good support for cell growth and proliferation, and the use of the dynamic bioreactor increased cell viability inside the scaffold thanks to a better diffusion of nutrients and oxygen inside the construct.
Conclusions: The conclusion of this study confirmed the advantage of using dynamic cultures culture conditions to fabricate 3D hepatic cells seeded scaffolds that could be used for the in vitro toxicity assessment of drug or contaminants.

Cardiovascular General 4: Cardiopulmonary – *GENERAL SESSION*

O64 (EI0208)

EXPERIMENTAL AND NUMERICAL RESULTS OF A NEONATAL OXYGENATOR WITH INTEGRATED PULSATILE PUMP AND HEAT EXCHANGER

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Objectives: Oxygenators are mostly used in combination with blood pumps and heat exchangers. To minimize the surface area and priming volume of the extracorporeal circuit these components can be combined. This could reduce the risk of adverse reactions.
Methods: Flexible silicone tubes (inner diameter 2 mm, wall thickness 150 µm) were placed symmetrically within the fiber bundle of an oxygenator. In combination with magnetic pinch valves at the oxygenators in- and outlet these tubes generate a pulsatile flow through the oxygenator by collapsing and expanding. The pulsating tubes actively distribute blood inside the oxygenators bundle. This could possibly increase the oxygenators gas exchange efficiency as it reduces the risk of shunt flows and recirculation- or stagnation areas. Currently, air is used to collapse and expand the tubes. By using tempered saline solution as driving fluid the tubes can be used as a heat exchanger also.
Results: Five in vitro tests were done following ISO 7199, using heparinized porcine blood. For flows up to 500 mL/min the oxygen and carbon dioxide transfer rates were 60-77 ml_{O2}/l_{blood} and 45-77 ml_{CO2}/l_{blood}, respectively. The test modules priming volume was below 25 mL incl. the integrated pump. Numerical simulations using ANSYS CFD and in vitro tests with a functional model prove the practicability of using the tubes as a heat exchanger.
Conclusions: Integrating a pulsatile pump within an oxygenator by means of collapsing and expanding silicone tubes is feasible as the in vitro tests show. The gas exchange was sufficient for the pumped blood flow in all five modules. Numerical and experimental results prove the practicability of using the silicone tubes in combination with tempered saline solution as a heat exchanger. This additional integration of a heat exchanger would further reduce the extracorporeal blood circuits' priming volume and potentially expand the range of applications for oxygenators.

O65 (EI0186)

PULSATILE OR CONTINUOUS CARDIOPULMONARY BYPASS: INFRARED THERMOGRAPHY USED TO SETTLE THIS DILEMMA

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Objectives: The debate about the possible advantages of pulsatile perfusion (PP) versus continuous perfusion (CP) in CardioPulmonary Bypass (CPB) is still open. Aim of this study was to prove the suitability of Infrared (IR) Thermography as a new methodology to compare the effects on peripheral perfusion exerted by PP or CP.
Methods: Patients (n=10) undergoing aortic valve replacement were randomized in two groups subjected to either CP or PP. An IR thermocamera was used to acquire temperature maps on the sole of the patient's left foot during surgery. Five reference areas were identified on the sole and temperature vs. time tracings were analyzed for each area. Continuous monitoring of the patient's central (*i.e.* rectal and esophageal) temperatures and of the arterial and venous blood temperatures was performed using 4 temperature probes during the entire surgical procedure. The heating process at the end of the hypothermic surgical phase was analyzed in terms of: time delay between the beginning of arterial blood rewarming and foot rewarming; slope of the temperature vs. time tracings during the heating phase and temperature differences among the five sole areas during the same heating phase.
Results: PP patients showed more prompt answer of the sole of the foot to arterial rewarming (shorter time delay) with respect to CP. The slope of the temperature vs. time tracings during the heating phase showed to be steeper in PP than CP. A more homogeneous temperature distribution on the sole of the foot was obtained in PP patients than in CP.
Conclusions: The IR thermography proved to be a suitable technique to evaluate peripheral perfusion during CPB. PP patients showed better peripheral perfusion than CP patients. This study is still ongoing, and a higher number of patients will be soon analyzed to confirm the outcomes obtained so far.

O66 (EI0053)

CLINICAL EVALUATION OF NEW-GENERATION OXYGENATORS WITH INTEGRATED ARTERIAL-LINE FILTERS FOR CARDIOPULMONARY BYPASS

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Objectives: New-generation oxygenators with integrated arterial-line filters have been marketed to improve the efficacy of cardiopulmonary bypass (CPB). Differences in designs, materials, coating surfaces, pore size of arterial filter and static prime exist between the oxygenators. Despite abundant preclinical data, literature lacks clinical studies.
Methods: From September 2010 to March 2011, 80 consecutive patients undergoing aortic valve replacement were randomized to CPB using Terumo-CapioxFX25 (40 patients, Group-T) or Sorin-Synthesis (40 patients, Group-S) oxygenators. Pressure drop and gas-exchange efficacy were registered during CPB. Fluid balance, ACT, INR, aPTT, fibrinogen, platelets, serum-albumin, total proteins, white blood cells (WBC), high-sensitivity C-reactive protein (hsPCR) were measured after anesthetic induction(T0), at CPB-start(T1), before CPB-discontinuation(T2), at ITU-arrival(T3), 3-hours (T4) and 24-hours (T5) postoperatively. The clinical outcome was recorded. Repeated ANOVA measure and non-parametric statistics assessed between-groups and during time differences.
Results: The two groups showed similar baseline and intraoperative variables. No differences were recorded in pressure drop, gas-exchange and acid-balance (p=N.S. for all) during CPB. Despite similar fluid balance (between-groups p=.979), Group-T showed lower serum-albumin (between-groups p=.014), total proteins (between-groups p=.0001), fibrinogen (p≤.004 at T4 and T5), platelets (between-groups p=.021), with higher INR (p≤.005 at T4 and T5), aPTT (between-groups p=.0001), hsPCR (between-groups p=.034), WBC (p=.003 at T5). Group-T also showed higher postoperative bleeding (p=.038) and need for transfusions (p=.0001). However, clinical outcome was comparable (p=N.S. for all clinical end-points).
Conclusions: Both oxygenators proved to be effective and resulted in

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comparable clinical outcome. However, Sorin-Synthesis seems to better preserve coagulative cascade and serum proteins, resulting in lower transfusions and post-CPB inflammatory response.

O67 (EI0054)

SELECTIVE PULSATILE LUNG PERFUSION WITH OXYGENATED BLOOD IN LOW RISK CORONARY ARTERY BYPASS PATIENTS IMPROVES PULMONARY HEMODYNAMIC AND RESPIRATORY INDICES

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Objectives: Pulmonary dysfunction after cardiopulmonary bypass (CPB) still accounts for high morbidity and mortality. The aim of this prospective study was to evaluate the effect of pulsatile pulmonary perfusion (PPP) with oxygenated blood during CPB on post-operative lung function and overall outcome.
Methods: Fifty low-risk CABG were prospectively randomized to receive PPP with oxygenated blood during CPB and aortic cross-clamping (25 patients, PPP-Group) or to conventional CPB (25 patients, control-Group). Pulmonary hemodynamic parameters [indexed pulmonary vascular resistances (PVRI), PAP, pulmonary capillary wedge pressure (WP), cardiac index(CI)] and respiratory indices [lung compliance (LC), PaO_2/FiO_2, alveolo-arterial-oxygen-gradient(A-aDO_2), mixed-venous-pO2(pvO_2)] were measured preoperatively, at ICU-arrival (T1), 3 hours postoperatively (T2), and post-extubation (T3). Broncho-alveolar lavage fluid was collected preoperatively, at ICU-arrival (T1-BAL) and after 4 hours. Clinical outcome was recorded.
Results: There were no differences in baseline variables and clinical outcome (p=NS for mortality, pulmonary morbidity, intubation time, ICU-stay). Patients undergoing PPP showed comparable pvO_2(p=NS at all time-points) but better postoperative LC (T1: 72.6±44.6mL/cmH_2O vs 31.0±6.9 control-Group:, p=.0001, T2: 78.9±55.5 vs 33.4±7.6, p=.0001, T3: 112.8±43.3 vs 77.9±26.1, p=.001), PaO_2/FiO_2 (T1:295.3±22.3 vs 235.6±72.4, p=.0001; T2:287.4±47.3 vs 232.2±32.4, p=.0001), with lower A-aDO_2 (T1:183.8±25.5 mm/Hg vs 225.4±61.1, p=.003; T2: 166.1±41.7 vs 254.7±33.3, p=.0001, T3: 96.0±33.1 vs 124.8±47.3, p=.016). CI (T1:3.1±0.3 l/min/m^2 vs 1.7±0.08, p=.0001; T2:3.3±1.0 vs 2.1±0.4, p=.0001; T3:3.6±0.6 vs 2.5±0.5, p=.0001), PVRI (T1: 296.8±99.8 dyne·sec·m^2/cm^5 vs 551.6±61.5, p=.0001, T2: 243.9±58.3 vs 312.0±23.6, p=.0001, T3:251.9±42.8 vs 295.9±27.8, p=.0001), WP (T1:8.4±3.3 mm/Hg vs 15.8±4.7, p=.0001; T2:7.8±1.9 vs 10.4±2.2, p=.0001) and PAP (T1:19.2±3.3 mm/Hg vs 27.4±4.5 p=.0001; T2:19.9±3.3 vs 25.4±2.5, p=.0001; T3: 16.5±3.1 vs 21.0±1.9, p=.0001) proved better in PPP-group. T1-BAL demonstrated lower neutrophils (79.3±3.8% vs 85.0±4.1%,p=.0001) and higher monocites (15.0±7.2% vs 10.1±1.4%,p=.001) in PPP-group.
Conclusions: PPP with oxygenated blood during CPB does not impact clinical outcome but significantly improves pulmonary hemodynamic parameters and respiratory indices in low-risk CABG.

O68 (EI0103)

PHYSIOLOGICAL TARGET CONTROL IN LONG-TERM EXTRACORPOREAL OXYGENATION

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Objectives: The application of long-term extracorporeal lung assist is still limited in clinical practice. Apart from long term material issues of oxygenator and blood pump also the usage scenario itself poses major implications on the design of the whole treatment system. While in the OR such machines are continuously operated by dedicated personnel, in the ICU operation and supervision are only intermittent or triggered by alarms. Especially the "easy" task of setting the appropriate machine parameters requires continuous readjustment of machine operating-values to patient demand and condition. In order to achieve this, we developed a closed loop control system which enables the direct control of physiological target values.
Methods: A fully computer controllable ECLA system featuring a centrifugal blood pump and an electronic fresh gas mixer was set up. Additional measurement equipment for blood flow and on-line blood gases was integrated into the external circuit and supplemented by an extended patient monitoring. A cascaded control scheme was developed and implemented on a DSPACE real time control system. The inner control loop is used to control the oxygenator output gas concentrations. This enables the independent control of O_2 and CO_2 gas transfer. The outer control loop then uses this to control physiological target values of venous CO_2 and arterial O_2 saturation.
Results: The control scheme was tested in an animal trial study of 8 pigs. By applying hypoxic gas concentrations at insufficient levels of minute ventilation, lung failure was simulated. Our physiologic control was tested at different levels of simulated lung failure. During 90% of the time target values could be kept within close boundaries.
Conclusions: Physiological target control is one of the key issues in treating lung failure with long-term ECLA. We could show the feasibility of our approach and its robustness against disturbances.

O69 (EI0005)

HEART LUNG MACHINE AND EXTRACORPOREAL LIFE SUPPORT: A MODULAR SYSTEM

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Objectives: Today, devices for Extracorporeal Circulation (ECC) like oxygenators and heat exchangers are limited to few different sizes. A total system replacement is performed for troubleshooting device complications as well as for switching from Heart Lung Machine (HLM) to Extracorporeal Life Support (ECLS), even though the components are partially identical (blood pump, oxygenator, heat exchanger). Therefore, we follow an entirely new concept, designing a modular system for: 1. An ECC adaptable to different patient sizes from neonate to adult. 2. A component-wise module exchange after complications. 3. The ability to switch from HLM to ECLS without total system replacement.
Methods: The system is modular regarding heat exchanger and oxygenator. It is adaptable to patient size and ECLS therapy by the number of modules. The pump blood flow is divided into the active number of parallel lines containing oxygenator modules and if required heat exchanger modules. A test circuit was set up for hydrodynamic measurements of pressure drop over the separated oxygenator and heat exchanger modules and of flow deviation to three parallel lines.
Results: Results of the hydrodynamic measurements show a total pressure drop over an oxygenator module and a heat exchanger module similar to a conventional oxygenator with integrated heat exchanger. The flow measurements demonstrate an equal flow through each parallel line.
Conclusions: The hydrodynamic measurements show the feasibility of a passive uniform flow deviation to parallel lines with heat exchanger and oxygenator modules. The pressure drop over the modules confirms the modular design concept. Currently, we are designing a first laboratory model of the ECC-system containing: inflow cannula, venous reservoir, rotary blood pump, several heat exchanger and oxygenator modules and an outflow cannula. Blood tests will be performed for the proof of concept.

Artificial Kidney Dialysis – *SYMPOSIUM*

K12 (EI0051)

DIALYZER INLET PRESSURE IS A LATE INDICATOR FOR FILTER CLOTTING DURING HEMODIALYSIS

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Objectives: A possible way to recognize thrombus formation within the capillaries of the hemodialyzer is to monitor the flow resistance by means of pressure measurements at the dialyzer inlet. When the dialyzer inlet pressure is measured continuously, trends of increasing flow resistance due to capillary occlusion could be predictive for developing coagulation. The purpose of this study is to assess the predictive value of dialyzer inlet pressure monitoring with regard to hemodialyzer clotting.
Methods: Dialyzer inlet pressures were measured in vitro during simulated hemodialysis treatments on a Fresenius 4008 with additional dialyzer inlet pressure sensor. During treatment the effective surface area of the capillary bundle facing the blood flow was varied by placing PVC rings of different sizes

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between the arterial hemodialyzer cap and the surface of capillary bundle. The arterial bundle surface area was varied between 0 and 90% in steps of 10%, and the corresponding increase in dialyzer inlet pressure was monitored.
Results and Discussion: According to the law of Hagen-Poiseuille, any increase in flow resistance in a bundle of cylindrical tubular hemodialysis capillaries should be mirrored by a pressure increase if blood viscosity and flow are constant. However, pressure increase in the post-pump arterial tube segment was below 5% for a surface area reduction of the hemodialyzer below 50%. A significant increase in dialyzer inlet pressure was only found if already 80% of all filter capillaries were blocked.
Conclusions: In principle, monitoring of dialyzer inlet pressures during therapy is a simple tool to identify patients at risk for thrombus formation. However, be aware that if you find a distinct pressure increase in the post pump arterial pressure segment, the filter has already gone most of its way to thrombosis. It follows that dialyzer inlet pressure is a late predictor for filter clotting.

O70 (EI0349)
WHAT ARE POSSIBLE REASONS FOR INFLAMMATORY REACTIONS OBSERVED IN PERITONEAL DIALYSIS?
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Objectives: Fluids for peritoneal dialysis may induce adverse clinical effects as reported in literature. We tested the hypothesis whether complement activation induced by polysaccharides through their hydroxyl moieties may contribute to these effects.
Methods: Six polysaccharides of different molecular weight were analyzed for their complement activating potential. Polymeric sugars, such as inulin, starch and Gluco-Pyranose-Polymer (Icodextrin®), oligomeric sugars, such as glycogen, and dimeric sugars, such as maltose and sucrose were used. 1. Polysaccharides were dissolved in saline and the resulting solution submitted to complement assays. 2. Polysaccharides were immobilized as adducts to activated human serum albumin at the surface of microtiter plates in order to simulate the adsorption of sugars to a solid surface, such as the peritoneal membrane. Complement-containing serum was added to the prepared sugar solutions or incubated together with albumin-linked sugars immobilized on MTPs. After incubation of the MTP at 37°C (1 hour) aliquots were collected and stored at 4°C. The formation of the stable split product C3a-desArg was assessed with the MicroVue Enzyme Immunoassay in triplets of aliquots.
Results: Complement activation is found with soluble as well as with immobilized polysaccharides. The C3a increment ranged from 12-60µg C3a/ml. C3a-generation is found in similar patterns in both experimental series. Both, biomaterials and polymers bearing hydroxylgroups are known to stimulate the activation of the complement system. Our investigations show that polymeric, oligomeric and dimeric polysaccharides are able to do so independently of whether they are dissolved or immobilized at a solid phase. Thus, all polysaccharides used in PD as osmotic agents may be involved in the stimulation of the immune system.
Conclusion: Evidence is provided that therapeutically used polysaccharides may induce the activation of the alternative complement pathway and may contribute to aseptic inflammatory processes seen in PD.

O71 (EI0206)
IMPROVING HEMODIALYSIS ADEQUACY OUTCOMES THROUGH CANNULATION PLANNING BY NUMERICAL SIMULATION
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Objectives: Cannulation is a key feature in hemodialysis as the hemodialyzer, but this procedure is much more empirical. Permanent access to an adequate blood flow is a requirement for successful hemodialysis session, where as repeated cannulation can damage the arteriovenous fistula (AVF). In this study, we propose to numerically model the blood flow in a side-to-end functional AVF and compare retrograde versus antegrade cannulation. We assess the hemodialysis adequacy by varying needle position and orientation.
Methods: In a previous work we published a 3D realistic numerical simulation of flow patterns and wall shear stresses of a patient specific AVF. In this study we added two back-eye needles to the venous site. A nephrology nurse prescribed a cannulation configuration, the needle position and orientation. Then we varied both parameters for the purpose of comparison. ICEM CFD and ANSYS CFX are used to mesh and solve the governing equations respectively.
Results: Numerical results showed abnormal blood circulation in the venous part of the AVF, caused by recirculation flow and low wall shear. Visualizing the blood flow streamlines with the needles revealed that particles from the arterial needle slipped back to the venous needle. Placing a needle against the blood flow exerted high venous pressure and possibly damaging the blood cells.
Conclusions: Proper cannulation of the AVF is essential to deliver adequate hemodialysis. There is disagreement whether needle orientation is or not associated with recirculation and/or in elevated venous pressure. This tool can be used in clinical practice for cannulation planning.

O72 (EI0107)
SAFETY AND EFFICACY OF HIGH CUT-OFF HEMODIALYSIS IN CHRONIC DIALYSIS PATIENTS: A RANDOMISED CONTROLLED TRIAL
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Objectives: High cut-off (HCO) hemodialysers are a new generation of dialysers with increased membrane permeability offering increased permeability to middle-molecules. We assessed the safety and efficacy of their use in a chronic dialysis population.
Methods: 29 prevalent stable hemodialysis patients were randomized to two groups (A/B) in a cross-over study: (A) received one HCO-HD (Gambro HCO 1100) and two standard HD sessions (Polyflux 170H) per week for 8 weeks, followed by 3x HCO-HD for 8 weeks; (B) received these treatments in reverse. Safety was assessed by serum albumin levels and adverse events. Patients were withdrawn if serum albumin dropped by >25% or a thrombotic event occurred. Efficacy was assessed by dialysis dose (Kt/V), removal of middle molecules, phagocytic functional test and pulse wave velocity.
Results: HCO-HD use thrice weekly caused a significant decrease in albumin (40.6g/L±3.4 to 36.7±2.6; p<0.01) over 4 weeks, however no further reduction was observed by week 8 (37.4±3.4), and no significant change occurred with HCO-HD used once weekly. No patients were excluded from the study for albumin loss. Four patients experienced adverse events; (x1) under-dialysed, (x3) clotted AVF. Mean Kt/V for HCO-HD was decreased compared to standard dialysis: 1.03±0.17 and 1.31±0.21 (p<0.05). Pulse wave velocity was reduced during thrice weekly treatment. Polyclonal FLC concentrations were significantly decreased in patients receiving HCO-HD thrice weekly (median decrease 18.17% (-139.20–56.47)) compared to once weekly (median decrease -5.50% (-85.76–14.24); p=0.008). Phagocyte function increased over the thrice weekly period from 91.2% ±9.4 to 98.1% ±2.1 (p=0.02).
Conclusions: HCO-HD offers clinicians the possibility for increased removal of larger uraemic toxins. Importantly, albumin loss plateaus by week 4 and was tolerated by all patients. Further work now needs to be undertaken assessing larger surface area HCO-dialysers and clinical surrogates should be studied in a larger population.

O73 (EI0062)
IN SPITE OF POSITIVE CHARGE ON POLYETHYLENEIMINE, AN69 ST MEMBRANE DOES NOT TIGHTLY ADSORB HEPARIN DURING CONTINUOUS RENAL REPLACEMENT THERAPY
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Objectives: We compared the effects of priming with different heparin doses (5000 IU/L in group A vs. 20000 IU/L in group B) on filter life span and systemic coagulation parameters in critically ill patients with acute kidney injury in this randomized cross-over study.
Methods: Different doses of heparin were randomly assigned to 24 patients [M:F = 18:6, median of age 68 (range, 50~96 years)] at the 1st and 2nd filter during renal replacement therapy (RRT).
Results: There was no difference of median values in baseline hemoglobin [9.1 (7.1~13) g/dL vs. 9.4 (8.3~11.2) g/dL, p=NS], platelet count [110500 (37000~352000)/mm³ vs. 108500 (23000~281000)/mm³, p=NS], activated partial thromboplastin time [aPTT, 37.9 (27.2~180) sec vs. 35.9 (27.2~97.2) sec, p=NS], prothrombin time [PT, 1.26 (0.98~2.09) INR vs. 1.19 (0.97~1.86) INR, p=NS], collagen/epinephrine clotting time [216.5 (104~300) sec vs. 223.5 (42~300) sec, p=NS], APACHE II scores [24 (7~34) vs. 24.5 (10~39), p=NS] and filter life span [15 h 57 min (5 h 5 min~71 h 47 min) vs. 17 h 18 min (3 h 40 min~71 h 32 min), p=NS] between two groups. Compared with baseline value of aPTT, its prolongation did not appear in 30 minutes after starting RRT in group A [from 37.9 (27.2~180) sec to 38.4 (25.3~180) sec, p=NS]. However, aPTT significantly increased in group B [from 35.9 (27.2~97.2) sec to 38.9 (29.7~86.8) sec, p=0.017] without clinical events.

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Conclusions: Priming with the higher dose of heparin and washing did not reveal the beneficial effect on filter life but prolonged aPTT. It suggests that polyethyleneimine does not strongly enough bind heparin and requires heparin-coated filter where heparin can be adhered more tightly.

Tissue Engineering of Skin: creating a new bio-artificial organ for clinical application – *SYMPOSIUM*

K13 (EI0433)
TISSUE ENGINEERING OF SKIN: CREATING A NEW BIO-ARTIFICIAL ORGAN FOR CLINICAL APPLICATION

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Large full-thickness skin defects resulting from burns, soft tissue trauma, congenital giant nevi, tumor resection, and disease leading to skin necrosis, represent a common and significant clinical problem that is far from being solved. The main challenges encountered are the following two: First, there is donor site shortage for autologous skin transplantation when the defect exceeds 50–60% of the total body surface area. The typical clinical example is a massive deep burn. Second, most conventional skin grafting techniques to provide autologous coverage are based on transplanting split-thickness skin (the today's gold standard). Split-thickness skin contains all of the epidermis but only part of the dermis, and that frequently leads to scarring. Rarely, scarring is mild and irrelevant. Often, particularly in children, there is hypertrophic scarring or keloid formation that is frequently disabling and disfiguring. There are still two major challenges concerning the development of novel skin substitutes: 1) On its way to an optimized and long lasting structure and function, a dermo-epidermal substitute has to be efficiently and appropriately vascularized. Attempts to reach this goal have entered a period of significant progress; however, a final breakthrough is still missing. 2) Much is still unknown about the mechanisms by which tissues form and heal, yet insights from developmental biology and other biological disciplines are already guiding the development of "instructive matrices" that work with nature's own mechanisms of organogenesis and repair. Biologically active matrices containing cells that constantly produce a physiological set of biologically active factors, in their appropriate concentrations and locations, in combination with secure, automated and highly reproducible techniques to produce a new generation of complex skin substitutes both, in a desired number and in a constant quality, are now the guidelines of modern "skingineering".

O74 (EI0416)
SINGLE EPIDERMAL-DERMAL SCAFFOLD FOR THE REGENERATION OF FULL-THICKNESS SKIN DEFECTS

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Objectives: Create a single tridimensional epidermal-dermal scaffold, using the spray-assisted Layer-by-Layer (LbL) to build a film on the top of a hyaluronan (HA) porous scaffold.
Methods: The porous scaffold was created by mixing modified HA in the aldehyde and in the hydrazide forms, followed by freeze-drying. A film, which is intended to act as an epidermal membrane, was produced on the top of this scaffold by sequential deposition of HA and Poly-L-lysine (PLL), creating a polyelectrolyte multilayer. The film build up mechanism was studied by spraying the electrolytes on silicon wafer at pH 5, 6 and 7 up to 50, 100 and 150 bilayers (BL). The topography and roughness of the films was determined using Atomic force Microscopy (AFM), and its thickness by profilometry. The attachment and proliferation of human keratinocytes (hKc) on the films was observed under SEM and its metabolic activity measured over time.
Results and Discussion: The films' thickness increased with the number of BL deposited, independently of the pH. The film created on the silicon wafer for 150 BL at pH 7 had a thickness of 1682.9±291 nm and a roughness of 1.11±0.38 nm. Also considering pH 7 as the most compatible for cell culture, this condition was selected to create the membrane on the top of the HA porous scaffold, in which hKc were able to attach and form a monolayer after 96 h of culture.
Conclusions: A one step method to produce a single epidermal-dermal scaffold was established by spraying the $(PLL/HA)_{150}$ polyelectrolyte on the top a porous scaffold. The membrane, physically connected to the scaffold, allowed its colonization by hKc. This strategy confers cohesion to the epidermal-dermal substitute, and is expected to contribute to improved performance of the skin substitute by promoting the interaction between the cells present in both layers.

O75 (EI0365)
BOOST OF EPIDERMAL STEM CELLS FROM ADULT KERATINOCYTES

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Objectives: Skin Tissue Engineering has the longest history of commercialization, however, skin analogues still lack in completely meeting the demands, especially in the cases of massive skin loss. The long-term function of the skin equivalents could be limited by the terminal differentiation of the grafted keratinocytes. Thus, the use of stem cells for this purpose, namely Epidermal Stem Cells (EpSCs), would provide an active source of biological material. EpSCs isolation difficulty remains, mainly due to the lack of well-determined approaches and markers. This work integrates an assemblage of strategies to be pursued in order to accomplish enrichment of this multipotent fraction.
Methods: Human primary keratinocytes (hKC) were isolated from human adult skin, and different methods for EpSCs fraction enrichment were applied. Rho-Associated Protein Kinase (Rock) Inhibitor Y- 27632 was firstly administered to freshly KC cultures to increase EpSC number. Consecutive selective methods, rapid adherence to b1-integrin ligand in collagen type IV and immunomagnetic separation methods, were then performed to establish populations based in the a6/CD71 expression. CFUs assay, flow cytometry and immunocytochemistry were then performed, focusing on the effect of the treatments over expression rate of early epidermal markers keratins19/5/14 and correlated with a6/CD71 sub-populations.
Results and Discussion: Collagen IV treatment resulted in increased cell adhesion, and polygonal shape and small size cells. Rock Inhibitor, not only enhanced cell proliferation (k14+ cells), namely the keratinocyte stem cell fraction $a6^+/CD71^-$, but also raised the expression of K19. Additionally, a boost in the keratinocyte stem cell fraction $a6^+/CD71^-$ with the expected morphology and in higher cell number, particularly in the fraction that was subjected to the previous treatments, was observed after the subsequent CD71 and a6-integrin immunomagnetic selections.
Conclusions: The methodology presented in this work indulges the boosting of EpSCs in hKC culture, with a consecutive purification and separation from hKC bulk.

O76 (EI0308)
DEVELOPMENT OF A VASCULARIZED SKIN EQUIVALENT

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Objectives: Due to the lack of an analogue for the vascular system, current skin equivalents (SE) cannot be used to test the capacity of a given substance to penetrate through the skin into the bloodstream. However the integration of a vascular system into a SE would amplify the possible applications in research fields such as toxicity testing or oncology, by providing a model for the critical barrier between the skin and the vascular system. The objective of this study is to integrate a full thickness SE into a biological vascularized scaffold (BioVaSc), based on an acellularized part of a porcine jejunum. This BioVaSc can already be used for the formation of renal and liver tissue and was successfully implanted into a patient as a trachea patch.
Methods: Primary human keratinozytes and human fibroblasts were seeded on the BioVaSc and cultured under submersed conditions for seven days. To initiate the differentiation of the keratinocytes, the construct was subsequently cultured at an air-liquid interface for another 12 days. The formation of skin tissue on the vascularized scaffold was determined using hemalaun/eosin (HE) and immunohistological staining.
Results: Histological HE and immunohistological staining revealed a stratified epidermal layer of keratinozytes with a corneous layer on one top of the BioVaSc and equally distributed fibroblasts inside of the scaffold. Thus we could show that the BioVaSc provides a suitable microenvironment that facilitates the

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formation of a functional SE.
Conclusions: In this work we demonstrated that the BioVaSc is a suitable scaffold for a SE. In future experiments we will combine the vascularized skin substitute with a new developed bioreactor that enables the supply of the vascularized skin substitute through the vascular system and the culture at an air-liquid interface.

O77 (EI0080)
ENGINEERING A FUNCTIONAL MICROVASCULATURE WITHIN A DERMO-EPIDERMAL SKIN SUBSTITUTE
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Objectives: The development of rapidly and efficiently vascularized tissue grafts is vital for tissue engineering. Especially within the first days after transplantation, rapid establishment of an intact vascular network and blood flow often decide whether or not a graft is taken. One way to accelerate the vascularization of an enginered tissue is to provide it with a pre-formed vascular network. The fundamental idea behind this approach is that fast anastomosis of a pre-formed vascular network with the patient's vascular system can compensate for the delayed neovascularization, which usually results in a shortage of blood supply right after transplantation. Here we describe the generation of an engineered skin substitute, from a single human skin biopsy, displaying a network of functional and anastomosing capillaries.
Methods: Primary human endothelial cells, fibroblasts and keratinocytes were isolated from human skin biopsies and expanded in vitro. These cells were used to generate a pre-vascularized dermo-epidermal skin substitute based on fibrin hydrogels. To test the effect of pre-vascularization, the skin substitutes were transplanted on the back of immuno-incompetent rats. The quality of the engineered skin was evaluated by excising the grafts after different time-points and subsequent analysis.
Results: The microvasculature produced within an organotypic skin substitute consists of a high number of branching and continuously lumenized capillaries. After transplantation, anastomosis with the rat vasculature occured and the differentiation process of the in vitro generated microvascular structures continued by the attraction of mural cells, which are known to support stabilization and maturation of capillaries.
Conclusions: We show that a network of branching and continuously lumenized capillaries within a dermo-epidermal skin substitute can be produced in vitro, and that prevascularization of tissue substitutes (derived from fibrin hydrogels) is demanding but possible.

Cardiovascular General 5: Device & Biology – *GENERAL SESSION*

O78 (EI0372)
FIRST STEP TOWARDS THE GENERATION OF A VASCULARISED 3D - CARDIAC CONSTRUCT
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Objectives: *In vitro* generation of bio-artificial cardiac construct (BCC) represents a promising method for the repair of ischemic heart tissue. Deficiency of oxygen and nutrient supply in the centre of 3-dimensional tissues may be addressed by *in vitro* vascularization. Here we investigate whether tubular-like network built by endothelial cells in a solid BCC, could be supplemented by externally applied endothelial cells through the BioVaM (Biological Vascularised Matrix), to generate a potential perfusion system.
Methods: BioVaM were decellularized using a protocol established in our lab. GFP-labelled rat heart endothelial (RHE) cell line were infused into the venous and RFP-labeled RHE cells were infused into the arterial vessel bed of the BioVaM. BCC were generated with a mix of isolated rat neonatal heart cells, collagen I, and Matrigel and casted onto the BioVaM, which was already pre-seeded with GFP/RFP labelled RHE cells. These constructs were cultivated for 10 days, and thereafter prepared for morphological analyses, performed via Confocal Laser-Scan-Microscopy (CLSM). Crysections were analyzed using fluorescent microscopy.
Results: Using a CLSM both cell types, GFP/RFP labelled RHE cells, were determined within the 10 days cultivated BCC-BioVaM complex. RHE cells were repopulating pre-existing vessels of the BioVaM, but most of these cells could be found through the whole thickness of the BCC. A dense, highly organized tubular-like branching within the BCC resembled to a capillary network, where red-labelled RHE cells derived from arteries and green-labelled RHE cells from veins. Both cell types also connected to the $CD31^+$ endothelial cells being a cellular component of BCC.
Conclusions: Invasion and capillary-like formation of externally added GFP/RFP labelled RHE cells from the BioVaM vessels into the cardiac construct represent an important step towards the engineering of a functional perfusion system, and thus vascularized and well-organized thick cardiac construct.

O79 (EI0379)
CORONARY ARTERY CALCIFICATIONS AND CARDIOVASCULAR MORTALITY IN HEMODIALYSIS PATIENTS
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Objectives: Coronary artery calcifications are frequent in renal patients and are related to significant cardiovascular morbidity and mortality. The possible relationship between coronary artery calcifications and overall cardiovascular mortality was investigated in maintenance hemodialysis (mHD) patients.
Methods: Two hundred and five patients (105 males and 100 females, aged 59.85±12.77 years, on mHD since 62.30±55.00 months) were enrolled into the study. All patients underwent a single cardiac Multi-layer spiral computed tomography (MSCT). Calcium load was quantified according to the Agatston score (AS; Agatston AS, JACC 1990). According to AS patients were then stratified into groups 1 (AS=0), 2 (AS 1 to 400), 3 (AS 401-1000) and 4 (AS>1000). All patients were followed up between January 2003 and January 2011. Primary end-point of the study was mortality for a major acute cardiac event. Seven-year actuarial survival was calculated for patients of the four groups separately by Kaplan-Meyer equation. Patients who died for causes other than cardiovascular disease and transplanted patients were censored. The log rank test was employed to compare survival curves.
Results: One hundred and two patients (49.7%) died for cardiovascular disease during the follow-up. Seven-year actuarial survival was more than 90% for patients of groups 1 and 2, but failed to about 50% in patients of group 3 and to <10% in patients of group 4. Hence, patients with AS>400 show a significantly higher cardiovascular mortality compared to patients of with AS<400 ($p<0.0001$).
Conclusions: The pathogenesis of arterial wall calcifications involves apoptosis and an osteoblastic-like transformation of smooth muscle cells that induces the synthesis of bone matrix and a local chemotactic activation of mineralization processes within the vascular wall. The presence of extended coronary artery calcifications detectable with cardiac MSCT may be predictable of an elevated risk of cardiovascular mortality at least in mHD patients.

O80 (EI0294)
NOVEL PSEUDO-ANEURYSM MODEL – USING A BIODEGRADABLE POLYMER
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Objectives: Several experimental aneurysm models exist which are mainly used to test new interventional methods for obliteration and/or exclusion of the aneurysms. However, creation of a pseudo-aneurysm formation has not been described. We present a novel method to induce pseudo-aneurysm by implanting a biodegradable vascular prosthesis in the infrarenal rat aorta.
Methods: In 6 anesthetized rats a polydioxanone (PDO 2mm-ID; 15mm-length) vascular prosthesis was implanted end-to-end in the infrarenal aorta and compared to 6 control rats with ePTFE prostheses. After 3 weeks a panangiography followed by graft explantation for histologic assessment was performed. The widest diameter of the graft was compared to the size of the native aorta.
Results: One animal died of ruptured abdominal aneurysm on day 20, one animal did not develop an aneurysm and the four remaining showed an increase

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in diameter of 256%. Autopsy showed saccular pseudo aneurysms. SEM and histology revealed fragmentation of the prosthetic material due to degradation, covered by a fibrous capsule containing parietal thrombus. All ePTFE controls were patent and showed no aneurysmal dilatation, nor pseudo-aneurysms.
Conclusions: Fast degrading synthetic polymers such as PDO will dilate, rupture and form pseudo-aneurysms after a period of 3 weeks, if implanted in the abdominal rat aorta with systemic pressures. This new aneurysm model may be of interest for testing new interventional treatment modalities.

O81 (EI0142)
ACOUSTIC DETECTION OF INITIAL THROMBOSIS FORMATION IN A NOVEL HEART VALVE TEST RIG
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Objectives: Even with the use of anticoagulants, thrombosis is still one of the major problems of mechanical heart valves. While investigating flow-induced thrombus formation, initial aggregates have to be determined. Laboratory parameters and pressure curves were not able to detect initial thrombus formation in this test setup, therefore acoustic methods were implemented.
Methods: Minimally heparinized porcine blood was used in a novel test rig that mimics the left ventricular anatomy and hydrodynamic conditions for thrombosis enquiries with the Saint Jude Medical bileaflet valve. Opening and closing sounds were acquired using a hydrophone (Type 8103; Bruel&Kjaer) placed proximal to the aortic root. Sound signals were processed using PULSE (Type 3560B; Bruel&Kjaer) and MATLAB. First, parameters in time and frequency domain were generated and evaluated using artificial silicon thromboses placed at positions of concern. Parameters independent from working conditions were selected. A Naive Bayes Classifier and an Artificial Neural Network with three layers and 20 neurons were applied for thrombosis detection via pattern recognition. Six subsequent blood tests provided optimization of the classifiers.
Results: Naive Bayes Classifier showed a sensitivity of 85% for thrombosis detection at the initial blood test. Classifier optimization over 6 blood tests increased sensitivity to 95%. The Artificial Neural Network could not be optimized by the blood tests and achieved a sensitivity of 76%.
Conclusions: Detection of initial thrombosis formation in vitro via sound analysis has proven a successful tool. Naive Bayes Classifier was found to be the more sensitive classifier and can be implemented to other valves. Future work will focus on the development of methods to detect the location of thrombus.

O82 (EI0181)
AGE-RELEATED CHANGES IN BIOMECHANICAL AND MORPHOLOGICAL PROPERTIES OF TRANSGENIC PORCINE VALVE DEDICATED FOR THE USE IN TISSUE ENGINEERING APPLICATIONS
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Objectives: Because of the imbalance of the donor and recipient, the cardiac valves derived from pigs do not express the galactose 1, 3 galactose (-Gal) antigen, and are attractive source for the preparations of tissue engineered heart valve. Due to the differentiation of recipient there is a need for the preparations of different size valve prosthesis. The aim of the study was to estimate how the age and weight of the transgenic porcine can influence the morphological and biomechanical properties of the valve prosthesis and how the size of the bioprosthesis, dedicated for different recipient can be controlled.
Methods: The morphological and biomechanical properties of acellular aortic and pulmonary valve in relations to the age and weight of transgenic porcine were tested. The valve morphology was analyzed using H&E and Masson staining. Uninaxial tensile test was used to estimate the biomechanical properties of the examinated valve. The computer simulations based on Finite Elements Methods (FEM) was used to study the influence of the decellularizations procedure on the hemodynamic conditions.
Results: The differences in the morphology and biomechanical properties of the acellular pulmonary and aortic valve were observed. Uniaxial tensile test demonstrated that the Energy to Break, Peak Load or Peak Stress of the aortic valve increase in relation to weight and age. For the pulmonary valve, the value of these parameters was constant, they were independent of weight and age of the animals. The value of biomechanical parameters was significantly lower for the pulmonary valve compared with the aortic valve.
Conclusions: The aortic valve derived from transgenic porcine is more valuable for the preparations of different size bioprosthesis**.** The use of tissue engineered pulmonary valve as an aortic valve replacement can be strongly limited.

O83 (EI0006)
IN-BODY BLUE LIGHT ILLUMINATION REALIZED THE FORMATION OF FUNCTIONAL AND ROBUST "BIOTUBE" VASCULAR GRAFTS WITH MANY CAPILLARIES AND ELASTIC FIBERS
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Objectives: The autologous "biotube", developed by using in-body tissue architecture technology, is one of the most promising small-diameter vascular grafts in regenerative medicine. The walls of the biotubes obtained by a traditional silicone mold-based method were very thin, and this is still the primary obstacle while handling anastomosis, even though these biotubes have adequate mechanical properties. The aim of this study is to evaluate the effect of optical stimulation of subcutaneous tissue formation in the body during the preparation of the biotubes.
Methods: A blue light-emitting diode (LED) and a small battery were embedded into a silicone rod as a luminescent mold (diameter 5 mm; length 50 mm). The biotubes were prepared by placing the molds into the dorsal subcutaneous pouches of beagle dogs (each weighing about 10 kg) for 2 weeks with initial 2-days illumination.
Results: The wall thickness of the obtained biotubes was 506.9±185.7 μm, which was about 7 times thicker than that of the previous biotubes prepared by 2 months of placing silicone molds without illumination. Smooth muscle specific α-actin positive cells migrated in the wall and many mature capillaries with smooth muscle cells were markedly observed in the middle layer of the wall. Very interestingly, the formation of elastic fibers was firstly observed only at two weeks along with collagen fibers mostly with a regular circumferential orientation. The resulting optical-stimulated biotubes could be auto-implanted in the carotid arteries without any stress in handling the anastomosis as well as the native one.
Conclusions: The short-term in-body optical stimulation resulted in the formation of robust biotubes with vascular components of smooth muscle cells and elastic fibers in addition to collagen fibers.

Citrate anticoagulation - a future option for extracorporeal blood purification - *SYMPOSIUM*

O84 (EI0436)
THE USE OF CITRATE-CONTAINING DIALYSATE FOR ANTICOAGULATION IN HEMODIALYSIS (HD). REPORT OF CLINICAL EXPERIENCE
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The blood membrane interaction induced during hemodialysis (HD) activates several cascade systems including the coagulation system, inducing clotting. To prevent from such clotting and to keep patency of the dialyzer, anticoagulation using heparin or low molecular weight heparin (LMWH) such as tinzaparin is used. As an adverse effect, the use of these anticoagulants, will increase the risk of bleeding. To avoid this, various settings are available such as the administration of citrate either intravenously or by local administration at the site of the dialyzer. The latter technique has been used for some years in the USA, while there is less experience in Europe. In this clinical setting 15 patients were randomized to a cross over using either LMWH-tinzaparin and a series of halved dose of tinzparin and local citrate anticoagulation using Citrasate (Cit), provided by Scandinavian Medical (Kista, Sweden). The study included analyses of subjective patency, ionized calcium (iCa), Kt/V. During Cit-HD the iCa was significantly more reduced with prolonged time. The lowest iCa measured was 0.96mmol/L. The median iCa after 210 min of HD was for Cit-Hd 1.02 and for tinz-HD 1.16 (p=0.001). Patency of dialyzers was estimated as clear in 9/15, stripes of clotted fibers in 5 and a red filter in 1 HD. In a second series, after further reduction of tinzaparin, patency was clear in 2/11, stripes in 5 and red in 4, of which 3 HD had to be prematurely interrupted. In conclusion the data indicate that local citrate anticoagulation may help to reduce or eliminate the use of heparin or LMWH for dialysis. This may help in preventing the risk for bleeding, especially in the course of surgery. However, optimization of doses of anticoagulants together with Citrasate have to be individualized.

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O85 (EI0432)
WHY CITRATE ANTICOAGULATION WILL BE THE FUTURE ANTICOAGULATION IN CHRONIC HEMODIALYSIS
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Objectives: Cardiovascular diseases have a very high impact on mortality and morbidity of patients suffering from end-stage liver disease being under chronic hemodialysis therapy. It is well known and clinically proved that chronic and acute inflammations are processes which are responsible for cardiovascular diseases, especially related to arteriosclerosis. Especially in RDT patients treated three times a week by hemodialysis – inflammatory processes are more or less continuously activated by the use of extracorporeal circulation using materials which are responsible for activation of inflammatory processes.
Methods: Citrate anticoagulation is a possibility to diminish or even completely inhibit those inflammatory processes during hemodialysis in case of using the optimal concentration of ionised calcium in the extracorporeal circuit. Using this optimal target concentration there is a possibility to inhibit the activation of the alternative pathway of the complement system. Furthermore, by complete inhibition of the activation of the coagulation system any link to other inflammatory systems like complement systems or even kinin systems are blocked. Additionally, there is also the possibility to block the release of microparticles from endothelial cells or even blood cells like platelets or white blood cells, which are also involved in inflammatory processes being responsible for genesis of arteriosclerosis.
Results: By analysing relevant literature it will be shown that by blocking any kind of inflammatory processes during hemodialysis there is a real chance to diminish cardiovascular complications in RDT patients and, therefore, to decrease the mortality but also morbidity of this patients.
Conclusions: Therefore, citrate anticoagulation should also be considered as a future anticoagulation method in ESRD-patients treated with hemodialysis regularly.

O86 (EI0431)
TARGETING IONIZED CALCIUM - AN ESSENTIAL TOOL IN CITRATE ANTICOAGULATION
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Objectives: Citrate anticoagulation (CA) is an upcoming method of anticoagulation (AC) in extracorporeal blood purification (ECBP). It offers several advantages such as prolonged filter life time, suppression of the complement activation, circumvention of HIT (heparin-induced thrombocytopenia) and the limitation of anticoagulation to the extracorporeal circuit (regional AC). However, to achieve these advantages, the ionized Calcium (iCa) concentration has to be kept within a certain range and accurate monitoring is mandatory. Aim of this study was to show that target-oriented CA offers a flexible and safe AC for patients in ECBP.
Methods: In vitro as well as in vivo studies were carried out with different target concentrations for iCa. Activated clotting time (ACT) and complement activation (C3a) were measured and correlated with the iCa.
Results: Our results show an excellent correlation between ACT and iCa. The complement activation can effectively be suppressed at iCa concentrations of ≤0.2 mmol/L.
Conclusions: Target oriented CA offers a very high flexibility in ECBP and enables an individually adapted AC based on the patients' needs.

O87 (EI0347)
CITRATE ANTICOAGULATION FOR DIFFERENT HEMODIALYSIS PROCEDURES
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Citrate has many characteristics of the ideal anticoagulant for hemodialysis. The major issue preventing its wider use is the complexity of current citrate anticoagulation protocols. Since 1993 we have been using 4% trisodium citrate for hemodialysis, plasma exchange and continuous renal replacement therapy, prepared by our hospital pharmacy. Calcium-free dialysate is used, with sodium and bicarbonate concentration usually reduced on hemodialysis monitor. In the last years we have performed more than 3000 citrate procedures per year, approximately 9-10 citrate procedures daily. The majority, approximately 60% of citrate procedures are performed in chronic hemodialysis patients, the rest of them acute patients, mainly in intensive care units. In chronic hemodialysis patients, citrate anticoagulation is usually performed for a limited time, when increased bleeding risk exists. However, in our center we have treated 16 patients with long-term (>3 months) citrate anticoagulation, with a maximum duration of 6.5 years, without significant side effects. Besides standard bicarbonate hemodialysis we have successfully used citrate for single-needle hemodialysis, predilutional on-line hemofiltration and plasma exchange. Special protocols were designed for each procedure. The use of calcium-containing dialysate was associated with significant clotting in venous bubble trap, both during hemodialysis as well as hemodiafiltration, despite higher citrate dose. Predilutional on-line hemofiltration was the only procedure with calcium-containing infusate (1.25 mmol/L) and successful regional citrate anticoagulation. In the last year we have successfully used citrate for prolonged, 8-hour high cut-off (Theralite, Gambro) hemodiafiltration in patients with plasmocytoma or rhabdomyolysis. If citrate accumulation and/or alkalosis occur, we perform short heparin-free dialysis to remove citrate and correct alkalosis. Despite its complexity, citrate anticoagulation is safe if performed by trained nurses and precise protocols. It offers many advantages over other anticoagulation methods. We can expect an increase in the use of citrate in future both in intensive care units as well as in chronic hemodialysis patients.

LATEST ADVANCES IN PREVENTIVE AND REGENERATIVE MEDICINE TECHNOLOGIES - *SYMPOSIUM*

K14 (EI0413)
INSTRUCTIVE MEMBRANES FOR NEURONAL REGENERATION
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Many strategies are aimed to develop biomaterials for supporting and inducing neuronal regeneration. Polymeric semipermeable membranes are attractive for their high selective properties for creating the microenvironment in order to promote neuron adhesion and growth. Micro- and nano-structured membranes would be able to modulate the adhesion, proliferation and differentiation of cells, which are fundamental processes for tissue regeneration by governing the mass transfer of molecules that generate a precisely controlled microenvironment mimicking the specific features of in vivo environment. Membranes may guide the axon regeneration with the surface geometry by controlling the mass transfer of molecules between the cell microenvironment and the external milieu, providing to the cells chemical, physical and topographical features similar to those of the complex in vivo extracellular matrix through patterns of chemistry and topography from macroscale to nanoscale. In addition, the membrane surface can be tailored with proteins, peptides and cell-specific recognition factors by modification processes in order to stimulate specific cell responses and maintain differentiated functions. In this paper the author will present the controlled design and preparation of polymeric membranes with appropriate physical, chemical and biological cues, which are relevant to induce the neuronal regeneration. In particular the influence of membrane configuration (e.g., flat, tubular), surface properties (e.g., roughness, pore size, porosity, topographical features), and physico-chemical properties (e.g., wettability) on neuronal outgrowth and differentiation as well as the membrane ability to reconstruct the neuronal network will be discussed. Neurite outgrowth and the orientation of cellular growth, which are two important processes, can be facilitated by designing a well-defined cellular pattern. Recent results in the development of synthetic and biodegradable membranes with tailored physical, chemical and morphological properties, which are engineered to stimulate neurite outgrowth, will be discussed.

K15 (EI0443)
SELF-ORGANZATION IN A CULTURE DISH
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Objectives: To develop a model of cellular self-organization in the laboratory.
Methods: Mouse embryonic fibroblasts (MEFs) cultured in 3-dimensional soft scaffolds. Real time PCR, western blot, immunohistochemistry.
Results: Cellular self-organization studies have been mainly focused on models such as Volvox, the slime mold *Dictyostelium discoideum*, and animal (metazoan) embryos. Moreover, animal tissues undergoing regeneration exhibit properties

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such as cellular de-differentiation and self-organization processes that ends in rebuilding tissue complexity and function. We speculate that the recreation *in vitro* of the biological, biophysical and biomechanical conditions similar to those of regenerative milieu could elicit the intrinsic capacity of differentiated cells to proceed to the development of regenerative structure. In this presentation I will show that when MEFs are cultured in a soft nanofiber scaffold they establish a cellular network that causes an organized cell contraction, proliferation and migration that ends in the formation of a tissue-structure that recapitulates certain aspects of early development, such as temporal control of early embryonic genes followed by tissue determination. Interestingly, a subset of early mesodermal genes (*Brachyury*, *Sox9* and *Runx2*) was up-regulated during this morphogenetic process. Interestingly, the expression of *Brachyury* determined the formation of an early mesoderm-like tissue followed in time by expression of Sox9 and Runx2, which resulted in the spontaneous formation of cartilage-like tissue.
Conclusions: Since cellular self-organization is an intrinsic property of tissues undergoing development this new experimental paradigm could bring new ways to obtain functional tissues in a dish.

Intra-aortic balloon pump as a cardiac assist device – *SYMPOSIUM*

K16 (EI0345)
SHAPE CHANGE OF THE INTRA-AORTIC BALLOON: CAN IT OFFSET THE OPERATIONAL DEFICIT AT ANGLES TO THE HORIZONTAL?
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Methods: Three balloons; cylindrical, 40cc, tapered increasing diameter balloon, 37cc (TiD) and tapered decreasing diameter (36cc TdD), have been compared in a mock loop at 0°, 20° and 30°. Pressure at 7 positions along the balloon, and flow-rate on either side of the balloon during inflation and deflation were sampled simultaneously at 2kHz. The ratios (R_{inf} and R_{def}) of water volume displaced towards and away from the tip of the balloon to the total volume displaced during inflation and deflation respectively, and time of maximum pressure (TMP) at each position were determined.
Results: R_{inf} for TdD and 40cc is decreased (51.28% vs. 44.96%) and (49.34% vs. 44.21%) respectively, while it is increased for TiD (49.26% vs. 50.14%) when angle changed from 0° to 30°. R_{def} decreased by 13.77% for TdD, 11.57% for 40cc and 5.12% for TiD when angle changed from 0° to 30°, respectively. TMP of TiD and 40cc balloons remained approximately unchanged with increasing angle; occurring earlier at the base than the tip of the balloon (TiD) and vice versa (40cc). TMP of TdD occurred almost simultaneously along the balloon at the horizontal position, increasing to a time-lag of 5ms and 12ms between tip and base with increasing angle to 20° and 30°, respectively.
Conclusions: R_{inf} and R_{def} of TiD are less affected by angulation than those of TdD and 40cc. TMP of TiD is also less affected by angulation compared to the 40cc and TdD. The shape of TiD appears to provide overall better inflation (R_{inf}) and deflation (R_{def}) benefits in vitro, which requires dimensional changes and in vivo investigation to establish the enhanced benefit of the newly shaped IAB.

O88 (EI0121)
EFFECTS OF BAROREFLEX ACTIVITIES ON IABP HEMODYNAMICS IN A CLOSED LOOP HYBRID CARDIOVASCULAR MODEL
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Objectives: Aim of this work is the integration of the autonomic mechanism of pressure regulation during temporary IABP assistance in a hybrid circulatory model.
Methods: The hybrid model is based on merging computational and hydraulic models. The lumped parameter computational model includes the upper thoracic aorta, circulatory districts (upper body, kidneys, splanchnic, lower body, pulmonary, coronary circulation) and left/right hearts. The hydraulic model provides a representation of the lower thoracic aorta by a silicon rubber tube containing a 40cc IAB. An additional numerical module provides a representation of the baroreflex mechanism in terms of afferent and efferent sympathetic nerve activity (ANA, ENA). Baroreflex model acts as a feedback control loop that regulates the blood pressure by changing heart rate (HR), peripheral resistance and venous tone of each circulatory district. Experiments were conducted applying IABP assistance to a pathological circulatory condition.
Results: The increment of diastolic pressure due to IABP provides an increment of ANA (+7%) and a decrement of ENA(-9%). Operating the IABP induced a reduction in HR by -6% (90 vs. 95 bpm), in kidney and upper body resistances by -5% (5.43 vs. 5.72 and 5.17 vs. 5.44 mmHg·s/mL, respectively). IABP also induced an increment in kidney flow by +7% (0.63 vs. 0.59 L/min) and upper body flow by +6.8% (0.50 vs. 0.46 L/min). By switching the IABP assistance frequency from 1:1 to 1:2 or 1:3 the mentioned effects reduce progressively. Results indicate that the short term effects of IAB are small, even in the presence of a model including baroreflex control.
Conclusions: The model provides an instrument for the assessment of IABP effects on baroreflex mechanism due to the increase of mean diastolic blood pressure. This contributes to predict and study the global evolution of hemodynamic condition after IABP activation and the resultant change in organ flows.

O89 (EI0187)
INTRA-AORTIC BALLOON PUMP: INDICATIONS FOR USE
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Objectives: The IABP has a long record in supporting patients after myocardial infarction or cardiac surgery. So far, the effects of IABP therapy have been investigated, but mainly in small observational studies and animal experiments, with conflicting results and variable magnitude of the results. Lack of well defined indications for use might explain these findings. To enhance its clinical efficiency and to better define indications for use, advanced models are required for testing the interaction of the IABP with patient specific factors of the cardiovascular system.
Methods: A patient having mild blood pressure depression (90/50 mmHg) and a lowered cardiac output was modeled in a lumped parameter model and a model-controlled mock circulation, both featuring a complete systemic, pulmonary and coronary vascular bed. The IABP was numerically modeled as a cylinder-shaped collapsible tube, while a real IABP was used in the mock circulation. IABP support was applied with the standard in clinical practice used timing settings, while the support capabilities of the IABP in terms of cardiac output, coronary flow, cardiac stroke work and mean aortic pressure were evaluated for different levels of ventricular contractility, heart rate and aortic compliance.
Results: Ventricular contractility, heart rate and aortic compliance appeared to be major determinants of IABP performance. IABP support showed more pronounced advantages in a clinical scenario of deep cardiogenic shock, than in a scenario of only mild blood pressure depression and a slightly lowered cardiac output. Increase in heart rate ultimately interfered with the time required for complete in- and deflation of the balloon, while a very distensible aortic wall permitted free wall motion in response to the augmented blood pressure.
Conclusions: The use of IABP therapy might be reserved for patients in deep cardiogenic shock, having a stiff aorta and a heart rate not exceeding 140 min^{-1}.

O90 (EI0057)
BALLOON-ASSOCIATED IMPACT ON THE PERFUSION OF VISCERAL ARTERIES AFTER THE INSERTION OF AN INTRA-AORTIC BALLOON PUMP (IABP)
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Objectives: The IABP is worldwide the most commonly implanted extracardiac, mechanical device in the treatment of patients with acute coronary syndrome and during cardiogenic shock. A CT scan confirmed malposition of the balloon–in this case an occlusion of visceral arteries–led us to start a systematic analysis of cardiac surgical patients. We analyzed the frequency of the malposition, its cause and the resulting clinical relevance.
Methods: From January 2007 to March 2009, a total of 621 of 7756 cardiac surgical patients (8.0%) received perioperative IABP support, of whom 63 (10.1%) received a thoracoabdominal CT during IABP support. Proximal and distal balloon positions were analyzed. The anatomic distance from the left subclavian artery to celiac trunk and aortic transverse diameter were measured and compared with implanted balloon dimensions. Mean age was 67.1±11.9 years; 33.3% were female, and height was 169±9cm.

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Results: Based on radiography, proximal balloon position was correct in 96.8% but only appropriate in 38.1% based on CT. In 61 out of 63 patients, compromise of at least 1 visceral artery was found: celiac trunk, 96.8%; superior mesenteric artery, 87.3% and renal arteries, 66.7%. Left subclavian artery to celiac trunk distance was 241±23 mm, and balloon length was 248±17 mm and corresponded to an anatomic balloon length mismatch in 68.2%. Spinal deformations were found in 42.9%. Laparotomy for mesenteric ischemia was required in 23.8%. Hospital mortality rate was 60.3%. **Conclusions:** In all of our patients the IABP was implanted according to the current implant procedure guidelines. In 96.8% of the patients, however, we found via CT scan a malposition of the proximal balloon end as well as an anatomic mismatch between aorta and the length of the balloon. For clinical reasons it is highly recommended to implant a shorter balloon length than the one which has been recommended so far.

O91 (EI0055)

HOW SHOULD I WEAN INTRA-AORTIC BALLOON PUMP? DIFFERENCES IN HEMODYNAMIC RESPONSE BETWEEN PROGRESSIVE "VOLUME-" AND "RATE-" WEANING

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Objectives: To evaluate the best method to wean a patient from intra-aortic balloon pump (IABP) after cardiogenic shock.

Methods: Thirty consecutive patients needing intraoperative IABP because of cardiogenic shock were enrolled in the study and randomized in the Intensive-Care Unit (ICU) to 2 different weaning protocols. Fifteen patients were randomized to be weaned by ratio (4 consecutive hours of 1:2 assisting ratio followed by 1 hour of 1:3 ratio; Group-R), 15 by progressive volume deflation (10 cc every hour for 5 consecutive hours; Group-V). Weaning protocol started if cardiac index >2.5 L/min/m^2, CVP≤12 mm/Hg, blood lactate <2.5mmol/L, mean arterial pressure >65 mm/Hg with a preserved diuresis of at least 5 consecutive hours. Five hours were "a-priori" set as weaning duration. IABP lasting >5hours was defined "failure". Pressure recording analytical method(PRAM) collected cardiac index(CI), indexed systemic vascular resistances(SVRI), and cardiac cycle efficiency(CCE) at 8 different time-points (T1 to T5 for the first 5 weaning hours, T6: 2 hours post-withdrawal; T7: 12 hours post-withdrawal; T8: ICU-discharge). Central venous pressure(CVP) at the same time-points, time from IABP-withdrawal to ICU-discharge and weaning failure were also recorded. Perioperative troponin-I and lactate leakage were compared. Repeated-measures ANOVA assessed group, time and group*time interactions.

Results: All patients were successfully weaned and discharged home. Group-V showed better preserved CI, CCE and CVP (group*time p=.0001 for all). Group-R had worse CCE since T3 to T8 (p≤.001), CVP since T4 to T8 (p≤.0001) and CI since T5 to T8 (p≤.0001). SVRI proved comparable during the entire weaning period (p=NS). Despite no differences were detected in troponin-I leakage, lactate proved lower in V-group since T5 to T8 (p≤.027). Time from IABP-withdrawal to ICU-discharge proved longer in Group-R (p=.0001).

Conclusions: Despite the quite similar clinical outcome, weaning the IABP by volume deflation after cardiogenic shock better preserved the hemodynamic parameters.

Artificial Organ Transplantation – *SYMPOSIUM*

K17 (EI0030)

LIMITATIONS IN CARDIOVASCULAR ORT

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Objectives: Cardiac and/or pulmonary transplantation is limited due to organ shortage, despite being the best long-term option. Therefore, major efforts are currently carried out to improve artificial or bio-artificial organs in the field of cardiovascular surgery. However, clinical results of small-calibre, synthetic vascular prostheses are still sub-optimal. Besides the engineering and power miniaturization, the main hurdles to overcome remain bio-compatibility, liability of infection and thrombogenicity. Our aim therefore is to develop a novel, synthetic, biodegradable tissue-engineered vascular prosthesis.

Methods: Cardiovascular patches and prosthesis have been developed by random nano-fibre electro-spun polycaprolactone (PCL) with and without drug additions and compared to ePTFE prostheses. *In vitro* tests included tensile, suture retention and cell growth. *In vivo* tests included thrombogenicity assays in the arterio-venous shunt (AVS) pig model, followed by vascular replacement in the rat abdominal aorta and in the pig carotid artery. Assessment included patency, compliance, thrombogenicity, cell ingrowth (endothelialization and matrix formation), as well as degradation.

Results: The *in vitro* mechanical properties and cell compatibility tests were better than ePTFE prostheses. *In vivo* thrombogenicity in the AVS showed an uptake of indium-labelled thrombocytes due to the porous structure. Long-term implants in the rat showed excellent patency up to 18-months with endothelialization, cellular ingrowth and matrix formation. However, calcification and regression of cellularity and angiogenesis can be seen beyond one year of implantation and in the pig model micro and macro-thrombus formation is seen at one month follow-up.

Conclusions: Despite the very promising manufacturing, mechanical *in vitro* testing and *in vivo* results up to one year, the biodegradable electro-spun PCL prostheses showed some late limitations, similar to the clinically-used ePTFE vascular prostheses, such as calcification and thrombus formation in cardiovascular applications.

O92 (EI0387)

THE ß AIR®: IMPLANTABLE BIO-ARTIFICIAL PANCREAS (BAP)

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Objectives: Current therapy for insulin-dependent patients requires frequent blood glucose testing and insulin injections. Quite often, this treatment does not result in optimized control over blood glucose. Islet transplantation could form the optimal solution for these patients once two key targets are met: adequate oxygenation and efficient immune protection of the donor tissue. The sub-dermal implanted βAir® is designed to overcome both hurdles.

Methods: The βAir® is a two compartment device. Donor islets of Langerhans immobilized in a flat sheet hydrogel and an oxygen chamber separated from the islet module by a gas permeable membrane. The immune barrier is a three-layer system: a 0.4µm hydrophilyzed Teflon membrane, that prevents cell-cell contact, thus preventing cellular immunity; a small pore size alginate hydrogel impregnated into the Teflon membrane, significantly inhibiting inward diffusion of immune macromolecules and a large flat alginate hydrogel inflicting with the capacity of cytokines and NO to affect the islets.

Results: The βAir® was implanted in small and large animals. Average blood glucose levels were adjusted to near normal for up to 6 months in iso and allogenic diabetic rats and for one month in large xenogenic pig. Upon retrieval, blood glucose levels returned to the disease state. No pig's DNA was found within the device. Migration of IgG across the impregnated membrane was reduced by 20 fold, suggesting a strict delay in penetration of antibodies and other components of the immune system. More than 90% of the alginate was maintained with minor loss of the cross linking ion, suggesting a stable alginate gel.

Conclusions: Results demonstrate the ability of the βAir® to treat diabetes in rats and pigs with long-term immuno protection.

O93 (EI0324)

MICROWELL SCAFFOLDS FOR EXTRAHEPATIC ISLET OF LANGERHANS TRANSPLANTATION IN TYPE 1 DIABETES

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Objectives: The conventional therapy for type 1 diabetes is insulin administration. Despite this, some patients are poorly controlled and suffer from hypoglycemia and long-term complications. For these patients, allogeneic islet transplantation into the liver has become an alternative therapy. Patients benefit from this therapy due to near normalization of blood glucose levels without an increased risk of hypoglycemia. However, islet graft function in the liver tends to decline over years indicating that the liver is not an optimal transplantation site. In order to develop alternative transplantation sites with better long-term outcome, we have developed a new microwell scaffold platform.

Methods: Microwell scaffolds were prepared from dense solution-cast and porous electrospun and salt-leached 4000PEOT30PBT70 block-copolymer

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films using microthermoforming. Polymer wettability and topology were assessed by captive bubble contact angle measurements, atomic force microscopy (AFM) and scanning electron microscopy (SEM). Furthermore, constructs were characterized for their permeability for the nutrient glucose. To determine the applicability of the constructs for islet transplantation, the morphology and function of human islets (three different donors) were studied after 7 days of culturing in the construct using SEM, histological analysis and glucose challenge tests.
Results: We fabricated reproducible dense and porous films. The polymer films were hydrophilic (contact angle 39°±2°). Diffusion tests revealed that electrospun and salt-leached scaffolds were permeable for glucose. Based on SEM and histological analysis there were no indications for islet spreading or outgrowth of islet stromal cells. Function tests revealed that human islets remained responsive to glucose challenge after 7 days of culturing in the constructs.
Conclusions: This study reports on the development of a novel microwell scaffold platform for extrahepatic islet of Langerhans transplantation. Alternative transplantation sites using biomaterial scaffolds may improve islet transplantation outcome.

O94 (EI0319)
IF THE PARAMETERS OF ENCAPSULATION OF LANGERHANS ISLETS INFLUENCE INSULIN SECRETION?
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Objectives: Diabetes remains a devastating disease, with tremendous cost in terms of human suffering and healthcare expenditures. A bioartificial pancreas has the potential as a promising approach to preventing or reversing complications associated with this disease. The immunoisolation of Langerhans islets have been developed as a method of normalization of the carbohydrate metabolism for diabetes. In this study we investigated the production of small diameter alginate microbeads (i.e.<0.3 mm) using electrostatic droplet generation. The aim of our study was to evaluate whether parameters of electrostatic droplet formation could influence the viability and secretory functions of Langerhans islets.
Methods: To investigate how the parameters of droplet generator have an influence on insulin secretion, hormone concentration was estimated during the encapsulated islets culture. Free, encapsulated islets were cultured for up to 10 days. The medium was changed every second day and the samples were taken and tested for insulin content. The islets in all tested groups were stained with dithizone and trypan blue before and after the culture.
Results: In all groups we observed that 100% islets exhibited insulin production (red dye). In one of the tested group the islets were stained with PI in 30% after 10-day culture. In the same group a decline of insulin secretion was observed to compare with control.
Conclusions: The viability and insulin secretion by encapsulated islets depend on the process parameters of their encapsulation.

O95 (EI0102)
ISLETS TRANSPLANTATION IN DIABETIC RATS: HUMAN ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS ENHANCE THE SURVIVAL AND INSULIN FUNCTION OF CULTIVATED ISLETS
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Objectives: Hypoxia plays a crucial role in pancreatic islet cell death during the culture period and in the first days after transplantation. Human mesenchymal stem cells (hMSCs) release antiapoptotic and angiogenic factors useful to protect islets during this period. The aim of this study was to evaluate the capacity of hMSCs isolated from adipose tissue (ADhMSCs) to protect islet viability and function in case of prolonged pre-transplant culture period.
Methods: Rat pancreatic islets were cultivated for 72 hours in absence (group 1) or presence (group 2) of ADhMSCs ($5x10^4$ cells/100 islets). At the end of the culture period islet cell viability was evaluated by histological analysis with immunofluorescence. Sub-therapeutical volumes of islets of the two groups (2000 ieq/Kg) were transplanted into the liver of streptozotocin-induced diabetic syngeneic rats (n=5 per group). Rats were subjected to daily measure of glycemia and, two weeks after transplant, to intraperitoneal glucose tolerance testing (IPGTT). A morphological evaluation of graft was assessed by immunofluorescence over hepatic lobes harvested after sacrifice.
Results: At the end of the culture period islets from group 2 showed a higher number of viable beta cells. Transplanted rats of group 2 presented a better glycemic control, detected by lower levels of blood glucose, than transplanted rats of group 1. IPGTT confirmed an enhanced islet function in group 2, which has resulted associated to an increased revascularization (larger number of lectin BS-1 positive cells) as highlighted by morphological analysis of the graft.
Conclusions: ADhMSCs seems to be efficient in protecting islet cell viability during culture period. The use of ADhMSCs is potentially useful in preserving islet functionality in case of prolonged pre-transplanted culture period.

New Biomaterials and Scaffolds – *SYMPOSIUM*

O96 (EI0376)
SUPERMACROPOROUS CRYOPOLYMERS FOR TISSUE REGENERATION
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Objectives: A range of porous synthetic and natural polymer hydrogels were produced using cryopolymerisation method and their potential as biomaterials for tissue regeneration was assessed.
Methods: The cryopolymers of polyvinyl alcohol, poly-HEMA, fibrinogen, collagen and gelatin were synthesized in aqueous solutions at temperatures below 273 K. The ice crystals were used as a pore-forming substance and the procedure led to formation of robust macroporous polymer structures capable of retaining their integrity upon multiple hydration-dehydration cycles. The pore structure of cryopolymers was characterized using cryo-NMR, confocal laser scanning microscopy, m-CT and SEM. Infiltration and proliferation of human dermal fibroblasts were used to study potential of cryopolymers as tissue scaffolds for wound healing.
Results: The porous structure of cryopolymers was comprised of fully interconnected (super) macropores in the range of 50-200 mm, suitable for cell migration and proliferation. In vitro results in a human skin model followed by experiments using large animal model showed high healing properties of the cryopolymers, which were better than in control experiments using commercial materials. Depending on the application, cryopolymers can be made biodegradable or stable, which depends on the degree of cross-linking and the nature of the polymer.
Conclusions: Cryopolymer-based tissue scaffolds have shown high efficiency in wound healing and potential for internal organ regeneration.

O97 (EI0304)
THREE-DIMENSIONAL HYDROGEL MIMICS HIERARCHY AND SIZE OF NATURAL FIBER BUNDLES
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Objectives: In many natural tissues, fibrils align in parallel and closely pack into three-dimensional (3D) hierarchical bundles of fibers. These fibers provide tensile strength to various tissues such as heart or bone. Given their importance in tissue function, engineering these hierarchical features into materials is of great biomedical relevance. Numerous strategies for the development of a synthetic fiber bundle have been proposed, such as extrusion of polymers into aqueous solutions. However, most of the existing techniques fail to replicate the hierarchical architecture of these tissues. Thus, the aim of this work was to engineer hydrogel fibers that both mimic the natural architecture of the fiber bundles and enable the encapsulation of cells.
Methods: Fiber bundles were fabricated by complexation between cationic chitosan (CHT) and anionic methacrylated gellan gum (MeGG) that occurred in a polydimethyl siloxane (PDMS) channel. Fibers were then collected and stabilized by photocrosslinking the MeGG. The resulting architecture of the fiber bundles was studied with atomic force (AFM), scanning electron (SEM),

transmission electron microscopy (TEM) and confocal microscopy. A closer system to biological matrices was achieved by covalently incorporating the adhesive motif RGD in the MeGG backbone.
Results and Discussion: Each bundle was approximately 100 µm in diameter and contained small fibers that were 1-5 µm in diameter. TEM revealed the structure at the nano-scale, exhibiting periodic gaps as in native collagen fiber bundles. Confocal microscopy of the hydrogel fiber bundles engineered with FITC-labeled CHT showed homogenous distribution of CHT throughout the fiber. Furthermore, encapsulated cardiac fibroblasts adhered to and spread along the fibril direction.
Conclusions: This system combines polyelectrolyte complexation and fluidics technology to engineer hydrogel fibers that closely mimic the natural architecture of fiber bundles at different scales. Given its simplicity we envision that it may be beneficial for various tissue engineering and regenerative medicine applications.

O98 (EI0038)
PRINTING OF A NEW GENERATION OF LOW TOXICITY POLYMERS BY ADDITIVE MANUFACTURING TECHNOLOGY FOR BONE TISSUE ENGINEERING
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Objectives: State of the art biocompatible and biodegradable poly(lactic acid) (PLA) has several disadvantages including bulk erosion mechanism, fast loss of mechanical properties, spontaneous release of acidic compounds and the inability to be structured by high resolution Additive Manufacturing Techniques (AMT). It was the aim of the current project to provide low toxic photopolymerizable formulations that can be printed by AMT to form 3D cellular scaffolds for bone tissue engineering with good biocompatibility and biodegradability.
Methods: Currently, most of the used photopolymers for AMT are based on (meth)acrylates. Beside the considerable irritancy and sometimes cytotoxicity of acrylate-based monomers, the formation of high molecular polyacrylic acid through hydrolytic degradation of the polymer is another undesirable aspect of these materials when applied to the biomedical field. Therefore, photopolymers based on vinylesters and vinylcarbonates as polymerizable group, which are FDA approved, low molecular and water-soluble poly(vinyl alcohol) upon hydrolytic degradation, were evaluated. Several monomers based on different substrates were synthesized to examine their cytotoxicity, photoreactivity, mechanical properties and degradation behavior. In vivo experiments of 3D-parts were carried out in New Zealand White Rabbits.
Results: In vitro studies with osteoblast-like cells, showed by far lower cytotoxicity than for their (meth)acrylate-based counterparts. By application of hydroxyapatite as filler mechanical properties already approached values from that of natural bone. The degradation behavior of the new polymers can be easily tuned between several months and years. In vivo studies showed excellent biocompatibility and osteoconductivity of the new materials.
Conclusions: It has been proven that the new generation of polymers have outstanding properties for the application in the biomedical field. Beside low cytotoxicity of monomers, polymers and degradation products, the polymers have tuneable mechanical properties. Furthermore, the degradation behavior can be tuned over a broad range and advantageous surface erosion mechanism (absence of acidic degradation products) can be seen.

O99 (EI0019)
PROPENSITY-MATCHED COMPARISON OF DRUG-ELUTING STENT IMPLANTATION AND CORONARY ARTERY BYPASS GRAFT SURGERY IN CHRONIC HEMODIALYSIS PATIENTS
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Objectives: Cardiovascular disease is an important cause of death in patients with end-stage renal disease, with approximately 20% of cardiac deaths attributed to acute coronary syndrome. However, the optimal management of coronary artery disease in hemodialysis patients has not yet been determined. This study compared the outcomes of coronary artery bypass graft (CABG) surgery and drug-eluting stent (DES) implantation in hemodialysis patients.
Methods: The study population consisted of chronic hemodialysis patients (dialysis duration > 6 months) with coronary artery disease who underwent DES implantation or CABG at the Asan Medical Center (Seoul, Korea) between January 1, 1999, and February 28, 2006. Primary end-points were major adverse cardiac and cerebral events (MACCE). Propensity score analysis was used to adjust selection bias and variable characteristics.
Results: Of 110 chronic hemodialysis patients with coronary artery disease, 44 underwent DES implantation and 66 underwent CABG surgery. After propensity score adjustment, the incidence of MACCE was significantly higher in the DES than in the CABG group (HR, 2.791; 95% CI, 1.155–6.746; p=0.023), but all-cause mortality did not differ between these groups (HR, 0.513; 95% CI, 0.095–2.773; p=0.438). Kaplan-Meier analysis showed that MACCE-free survival was significantly longer in the CABG than in the DES group (log-Rank p=0.023).
Conclusions: We found that, compared with DES, CABG significantly reduced the incidence of MACCE and target vessel revascularization (TVR) in chronic hemodialysis patients.

Modelling of Cardiovascular and Pulmonary function in regard to clinical applications - *SYMPOSIUM*

K18 (EI0440)
PHYSIOLOGICAL MODELLING IN COMPUTATIONAL DESIGN OF BLOOD-HANDLING DEVICES
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Modelling and computational analysis play an increasingly important role in bioengineering, particularly in the design of implantable ventricular assist devices (VAD) and other blood-handling devices. Numerical simulation of blood flow and associated physiological phenomena has the potential to shorten the design cycle and give the designers important insights into causes of blood damage and suboptimal performance. A set of modelling techniques is presented which are based on stabilized space-time finite element formulation of the Navier-Stokes equations. Specific issues affecting shape optimization in this setting, such as parametrization of complex 3D surfaces and sensitivity to constitutive model selection, will be discussed. In order to obtain quantitative hemolysis prediction, cumulative tensor-based measures of strain experienced by individual blood cells must be developed; red blood cells under shear can be modelled as deforming droplets, and their deformation tracked along pathlines of the computed flow field. An alternative continuum-based approach is also under investigation. Another aspect of blood pump performance is related to platelet aggregation and thrombus formation. A three-species model for platelet aggregation is being developed based on a set of physiological experiments in collaboration with the Aachen University Clinic.

O100 (EI0034)
A MODIFIED SULFITE SOLUTION MEASURING OXYGEN UPTAKE OF OXYGENATORS AS AN ALTERNATIVE TO BLOOD
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Objectives: Blood oxygenation is the main factor evaluating the efficacy of an oxygenator (artificial lung). In conventional calculation of oxygen transfer rate (OTR) *in vitro*, water and whole blood are used which consequences with inaccuracies and handling difficulties respectively. Therefore, a novel sulfite solution with modified concentrations of its components has been proposed, which demonstrates a similar behavior to natural blood in case of oxygen uptake rate (OUR) and simulates its oxygenation.
Methods: The modified sulfite solution comprises 0.5M sodium sulfite (acts as hemoglobin in oxygen uptake capability), 10^{-3}M cobalt sulfate (as catalyzer), 0.012M phosphate buffer (Na_2HPO_4/NaH_2PO_4) and 10^{-5}M bromothymol blue (as color indicator). The solution pH is adjusted at 8 with 30%wt sulfuric acid. To regulate the solution OUR to that of natural blood, 32mL of this solution should be diluted to 1000mL. This solution is then tested with a hollow fiber membrane oxygenator (Medos AG, GERMANY). Using flow rates from 500 to 2800 [mL/min], oxygen uptake in this solution follows with a quick reduction of pH to

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4, recorded by a sensitive pH sensor AZ8601, and is also indicated by color changes from blue to yellow.
Results: The modified sulfite solution demonstrated similar maximum OUR (201 [mlO_2/L]) to natural blood, when all sodium sulfite reacted with O_2 and converted to sodium sulfate while pH remains constant around 4. Moreover, OTR in different flow rates shows an access of ±7.6% to blood in *in vitro* tests, e.g. with flow rate of 1000 [mL/min], the calculated OTR is 67 while the one of blood is 65 [mlO_2/min].
Conclusions: This modified sulfite solution can substitute natural blood in OUR and OTR tests *in vitro*. Therefore, it can be used as a reliable alternative to whole blood evaluating the performance of oxygenators in research and clinical applications.

O101 (EI0224)
A NUMERICAL STUDY OF BLOOD FLOW BEHAVIOR IN BLOOD VESSELS WITH EMPHASIS ON THROMBOEMBOLIC COMPLICATIONS
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Objectives: Thrombotic events due to activation of platelets and plasmatic clotting factors can have fatal consequences. Especially patients who rely on vascular grafts or stents run an increased risk of developing thrombosis. Both the biomaterials and the flow properties in these conduits may trigger adverse platelet reactions. Blood flow conditions in arteries are characterized by Computational Fluid Dynamics (CFD). Furthermore, a mathematical model to describe thrombocyte reactions is presented and validated with respect to experimental data in an in vitro flow system modeling stenosed arterial flow.
Methods: A continuum approach to modeling of platelet activation, adhesion and aggregation is used. The method is based on the advective and diffusive transport of resting and activated platelets and platelet-released agonists. Parallel computing resources allow solving the equations on refined meshes. For the characterization of the role of different adhesive proteins, a shear flow configuration is used. Scanning electron and fluorescence microscopy are applied to evaluate platelet adhesion and visualize the binding proteins.
Results: The non-pulsatile and pulsatile flow through healthy coronary, aortic and femoral arteries was computed as well as the flow through an occluded coronary artery. The dependence of thrombosis on the local flow conditions could be shown experimentally. The role of the binding proteins, e.g., fibrinogen and Von Willebrand Factor as a function of the local shear rate could be determined.
Conclusions: Several arterial flow conditions and corresponding platelet behavior were studied both in vitro and numerically. The presented simulations are in good agreement with experimental data.

O102 (EI0209)
DEVELPMENT OF *IN VITRO* ACCELERATED FATIGUE TESTER FOR CORONARY STENT WITH A FUNCTION OF CYCLIC BENDING
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Objectives: As proximal right coronary artery (RCA) is reported as one of the most frequent sites of stent fracture, we focused on fracture potentials of five drug-eluting-stents (DES) platforms using our in vitro tester which can apply an in vivo simulated cyclic-bend load.
Methods: According to frontal coronary angiographic data of 63 patients, average bend angles of RCA between end-systole and end-diastole were analyzed to be 105° and 125°, respectively. The angle data classified in type-B referred to ACC/AHA guideline were used to fabricate a RCA silicone replica. Moreover, mechanical stiffness was adjusted to that of human coronary arteries. The accelerated durability tests were performed under the above cyclic bend environment. Cyclic bend-load was exerted 1200 times per minute to the stent installed in RCA replica filled with 37°C phosphate buffered saline. Fracture potentials of Cypher 316L stainless steel (SS) stent (3.0mm×18mm, Cordis), Taxus Express2 SS stent (3.0mm×20mm, Boston Scientific), Liberte SS stent (3.0mm×20mm, Boston Scientific), Driver cobalt alloy stent (3.0×18mm, Medtronic), and Multi-Link Vision L605 cobalt chrome stent (3.0mm×18mm, Abbott) were investigated (n=6 each). All tests were conducted for 10year-equivalent duration except for incidence of stent-separation.
Results: No fracture was observed in Driver stents, however, Cypher stents were all fractured and complete separation of the mesh occurred in 12±6 days equivalent (n=6). One Vision stent was completely separated in 1020 days equivalent, while 5 Vision stents had no fracture. One Liberte stent had one single strut fracture, while other 5 Liberte stents had no fracture. Strut fractures with partly separation were observed in Taxus Express2 stents, which were started in 178±310 days equivalent.
Conclusions: A novel test-platform to predict fracture potentials of clinically available stents was developed. The accelerated durability tester with a function of cyclic bending elucidated different incidences of stent fracture for five DES implanted at RCA region.

O103 (EI0182)
THE USE OF A LUMPED PARAMETER MODEL TO OPTIMIZE BIVENTRICULAR DEFIBRILLATOR PROGRAMMING: RESULTS OBTAINED BY A RANDOMIZED AND PROSPECTIVE STUDY ON 60 PATIENTS
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Objectives: Biventricular defibrillator (BIV) implantation is a succesfull treatment for chronic heart failure despite of 30% not responders, partly due to sub-optimal atrioventricular (AV) and interventricular (VV) intervals programming. Aim of this work was to develop a numerical model (NM) for AV and VV optimization.
Methods: A lumped parameter circulatory model was updated. Atria, ventricles and septum were described by variable elastance models and their mechanical activity was related to ECG. BIV was modelled as an impulse generator driving heart chamber contraction. 60 patients were enrolled and randomized into two groups (A, B). All patients were studied by Echo, ECG, pressure measurement, six minute walking and Minnesota tests before BIV, 1, 3 and 6 months (f-u1,3,6) later. In Group A, AV and VV were programmed and modified, if necessary, at each f-u by NM optimizing hemodynamic variables. Group B was programmed by standard algorithm optimizing QRS.
Results: There were no statistically significant differences between A and B at the baseline in QRS and left ventricular end systolic (LVes) and end diastolic volumes (Lved) (QRS:A:154.7±39.5ms; B:141.9±39.5ms; LVes:A:187.1±92.7mL; B:155.6±57.6mL; LVed:A: 246.1±101.2; B:226.1±67.1) and between simulated and measured data in A at the baseline and at f-u6 (p>0.3).
At f-u6, comparing A to B, it was observed:
- no statistically significant difference in QRS reduction (p>0.7),
- a positive trend in Six minute and Minnesota tests (p=ns) and in ventricular remodeling (A:ΔLVes=-56.6mL, ΔLVed=-53.7ml; B:ΔLVes=-28.1mL, ΔLVed=-22.45mL, p<0,05).

Conclusions: For each patient, the optimal AV and VV are different and can change at each follow-up. A personalized and dynamic therapy based on the developed NM could improve patients' outcome.

Artificial Kidney Dialysis Techniques - *SYMPOSIUM*

K19 (EI0174)
PRESENCE OF MICRO BUBBLES IN HEMODIALYSIS. PHYSICAL BASIS, TECHNICAL CONSIDERATIONS AND REGULATIONS
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Objectives: Micro bubbles have been observed in the hemodialysis extracorporeal circuit (EC). The origin of these micro bubbles is still debated. The purpose of this work is the elimination of several possible causes of bubble production.
Methods: Literature studies, calculations and photographic documentation.
Results: Degassing under the influence of negative pressure requires nucleation sites in blood. Numerous papers published between the 18th and the 21st century have demonstrated the absence of nucleation sites in venous (and arterial) blood. Sudden acceleration of the blood column may cause cavitation, as known from metal heart valves. Acceleration caused by blood pump pulsations is more than an order of magnitude below the critical limit. Diffusion of air from the dialysate side can be excluded even if air is visible on the dialysate side because of the partial pressure gradient. The likely origin of air bubbles is air remaining in the EC including the dialyser after priming or leaks in the pre-pump part of the EC, which is under negative pressure. Another source of air bubbles may be saline used for priming.

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Conclusions: The number of air bubbles reaching the patient from the EC can be reduced by careful priming. It is likely that priming by backfiltration or by on-line produced substitution fluid will result in considerably less air bubbles because dialysate is degassed and air bubbles remaining in dialyser pores may be expelled more efficiently during the priming phase.

O104 (EI0327)

EVALUATION OF AIR CONTAMINATION INCIDENCES AND *IN VITRO* SETTINGS AND EXPERIENCES OF MICRO BUBBLES

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Objectives: During hemodialysis air infusion may occur. In Sweden incidents occurred using different systems. Air could be introduced into the blood stream from couplings where, for example, a negative pressure is present besides residual air. The aim was to find a method that could be used to verify presence and size of air contamination, such as micro bubbles in dialysis extra corporal circuit.
Methods: *Qualitative:* A dextran and albumin (D-A) solution was developed to simulate blood that enables study of micro bubbles by visual inspection. *Quantitative:* Bubble detectors Hatteland CMD10, and EMX25 were used to count and verify size of bubbles. *Calibration:* The system was calibrated and validated using de-aired water and glass beads. *Measurement:* A set of blood lines was connected to a container with dextran-albumin solution that was re-circulated in the system. A bubble detector probe was attached to the system. One variable at a time was changed, such as flow or venous chamber. Statistic: paired nonparametric statistics were used.
Results: Visual inspection verified the presence of micro bubbles in venous lines. Calibration: There were no detected counts with de-aired water. There was a normal distributed count of glass beads with a range of a 9-graded scale (the size was calculated to be ≥45µm at level 9 at 0dB, down to 2.5 µm at 0dB at grade 1). In vitro tests showed that the micro bubble distribution correlate to flow, dialysator and shape of venous chamber.
Conclusions: The method using a D-A-solution is a fast qualitative method to get a view of bubble distribution in extra corporal systems. Together with a bubble detector and paired non parametric statistics it is a robust and effective method for in vitro testing to evaluate and compare bubble exposure in extra corporal systems.

O105 (EI0228)

***IN VITRO* TESTING OF PREVAILING MATERIALS AND INITIAL CLINICAL FINDINGS**

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Objectives: During HD previous studies have shown that especially micro bubbles of air may pass the air detector. These studies focused to analyse *in vitro* if the air trap of various producers may contribute to the presence of micro embolic counts in the fluid that has passed the air trap detector. In addition another *in vitro* study analysed if the dialyzer by itself may contribute to these contaminations. In parallel a clinical study was performed to evaluate if findings during in vitro tests could be found to some extent during chronic hemodialysis. If such contamination was present how frequent this would be and would it be a greater risk at the start or the end of dialysis.
Methods: A ultrasound probe was placed on the venous dialysis tube after the air detector and venous chamber (Hatteland, Norway).
Results: The studies verified previous in vitro studies with micro embolic counts that pass the air trap without inducing an alarm. Fewer embolic signals were detected in the in vitro studies when using a high level of the fluid in the air trap and when using a wet dialyzers. In the clinical studies high counts were present both at the first period as well as the last period of hemodialysis.
Conclusions: These studies verify the finding of embolic counts in various extents in various in vitro settings, but also verify the presence of such counts in the dialysis tubes after the air trap in clinical routine hemodialysis. Further studies are warranted to clarify how to prevent patients from such problems and to clarify if the counts are clinically relevant.

O106 (EI0112)

A HIGH BLOOD LEVEL IN THE VENOUS CHAMBER AND A WET STORED (GAMMA STERILIZED) DIALYZER HELP TO REDUCE EXPOSURE FOR MICRO BUBBLES OF AIR

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Objectives: Twenty long-term hemodialysis patients with an age ranging from 42 to 80 years (mean age: 65 years) were investigated. There were eight women and twelve men.
Methods: The patients were randomized to a cross-over of 3 modes of HD: Mode 1: F8HPS (Fresenius, steam sterilized) with a low blood level in the air chamber (FL); Mode 2: F8HPS with a high level (FH); Mode 3: Rexeed (Asahi Kasei Medical, gamma sterilized, wet stored dialyzer) with high blood level (RH). Micro bubble measurements were continuous during 180 minutes of HD for all settings. The conditions were the same for each patient throughout the series.
Results: A Multiple Poisson regression was used to test the effect of filter type on the amount of bubbles in the blood. There was a significant effect between different filter settings and bubbles detected in blood both for RH vs FL (OR 4.07, 95% CI 4.03-4.11, $p<0.0001$) and FH (OR 1.18, 95% CI 1.17-1.19, $p<0.0001$) and for FL vs FH (OR 0.290, 95% CI 0.288-0,293, $p<0.0001$). This means that bubble exposure is least when using RH, more with FH and most with FL.
Conclusions: During hemodialysis micro bubbles of air develop in the blood circuit in the device. These micro bubbles can pass the venous chamber and enter into the circulation of the patient. This study shows that using a high fluid level in the venous chamber results in a lower exposure for MB and by using wet stored dialyzers this exposure was even less. These cost-limited measures may have an important impact in reducing the MB exposure to the patient.

O107 (EI0232)

MICRO EMBOLIES OF AIR ARE DEPOSITED IN THE ORGANS IN HEMODIALYSIS PATIENTS. A CASE REPORT

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Objectives: HD patients (HDp) in comparison to peritoneal dialysis have an increased prevalence of pulmonary fibrosis and cerebral alterations. We previously showed that during HD the blood that passes the dialysis device gets loaded with micro bubbles of air (MB) that are returned to the patient without inducing an alarm. These MBs can be detected as embolic signals by an ultrasound device. These embolic signals increased significantly during HD, both in the AV-fistula and in the carotid artery (Forsberg et al. 2010). Aim: To clarify if these signals are due to micro embolies of air or blood or just artifacts, we perform histopathology of autopsy material of HD patients (approved by the Ethical Committee).
Methods: Our first results are from a 61-year-old man who was on chronic hemodialysis for 5 years, due to diabetic nephropathy. During an episode of pulmonary edema, due to fluid overload, he was ultrafiltered. Within half an hour after start he suffered from a cardiac arrest and died. Autopsy verified the clinical findings. Tissue was fixed and stained using antibodies to C3, IgG, IgM and fibrinogen
Results: Microscopic investigation of the lungs, brain and heart verified the presence of micro embolies of air that were surrounded by fibrin in these organs. The latter indicates that MBs were deposited before death occurred.
Conclusions: Autopsy data show that micro embolies of air enter the blood during HD in the dialysis device and are trapped in the lungs. In addition they pass the pulmonary capillaries and arterial part of the body and are dispersed throughout the whole body. These data strongly support that these MBs cause micro embolies and organ impairment and can be part of the bad prognoses found in HDp. Data also support the importance of reducing the extent of MBs in the dialysis circuit.

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Natural based polymeric biomaterials and composites for Regenerative Medicine - *SYMPOSIUM*

O108 (EI0396)

TISSUE ENGINEERED CONSTRUCTS FOR PERIODONTAL REGENERATION BASED ON ADIPOSE STEM CELLS AND A NEWLY DESIGNED POLYMERIC SCAFFOLD

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Objectives: Periodontal disease, highly prevalent in human and canine species, is an inflammatory pathology, which can result in tooth loss and in systemic implications. The current therapies are ineffective in avoiding the epithelium growth, bone resorption and ankilosis, which inhibit the formation of a functional periodontal ligament. Tissue Engineering (TE) strategies, combining scaffolds and mesenchymal stem cells, has paved the way for new therapies. Our goal is to develop an innovative construct for periodontal regeneration, based on culturing adipose stem cells (ASCs) onto a bi-layered scaffold comprising a starch+poly(e-caprolactone) (SPCL) membrane, which acts as a guided tissue regeneration membrane, and a SPCL fibre mesh functionalized with osteoconductive silanol groups.
Methods: The SPCL membrane was obtained by solvent casting and then combined with a wet-spun fibre mesh (WSFM) with/without silanol groups. Bi-layered scaffolds were characterized by scanning electron microscopy (SEM), tensile tests, Fourrier Transmission Infra-red (FTIR), and enzymatic degradation assays. Canine ASCs (cASCs) were obtained from subcutaneous adipose tissue harvested upon programmed surgeries. The proliferation of cASCs seeded/cultured onto the scaffold was studied by dsDNA quantification and SEM. Osteogenic differentiation on the WSFM was assessed by ALP quantification, real time RT-PCR (osteoblastic markers) and histology (Alizarin Red and Lévai Laczkó stainings).
Results: SEM revealed a good adherence between the layers, roughness and fibres interconnection. FTIR confirmed the presence of Si-O-Si and Si-OH bonds in functionalized WSFM. Also, the scaffold exhibited suitable mechanical properties and degradability to be applied in an *in vivo* environment. Culturing experiments showed that materials provide a good support for ASCs according to DNA increasing and SEM. ALP activity increased until 21th day and also the calcium content revealed osteoconductivity and bioactivity.
Conclusions: This work showed that cASCs onto this SPCL bioactive scaffold are a promising TE approach to reach periodontal regeneration, namely, in its osseous component.

O109 (EI0439)

NATURAL-BASED PHOTOCROSSLINKABLE POLYELECTROLYTE COMPLEX HYDROGEL: DEVELOPMENT AND MICROFABRICATION

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Objectives: When two oppositely charged polyelectrolytes are mixed together, a physical hydrogel is formed through molecular interactions. These interactions are reversible and can be disrupted by changes in the ionic strength, pH or temperature. To surpass this, we used a photocrosslinkable polyelectrolyte that enabled the stabilization and microengineering of polyelectrolyte complex (PEC) hydrogel.
Methods: Anionic methacrylated gellan gum (MeGG) was added to the cationic chitosan (CHT) solution in different ratios and exposed to UV light to form PEC hydrogels based on these natural polysaccharides. The chemistry of the surface and bulk of the hydrogel was analyzed by Fourier transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS). Transmission electron (TEM) and confocal microscopy showed the distribution of both polymers within the hydrogel. Microfabricated structures were produced by placing a photomask on top of the PEC.
Results and Discussion: The initial electrostatic interactions that occurred upon contact between the two polymers did not allow for an instantaneous mixing. Upon capsule formation, the mixing of both polymers proceeded slowly. TEM and confocal of fluorescein-labeled CHT suggested the migration of CHT to the interior of the apparent MeGG capsule. FTIR and XPS chemically validated these findings. The photocrosslinkable feature of MeGG further enabled the formation of a number of micro-units with different shapes and sizes with viable encapsulated fibroblasts.
Conclusions: We successfully fabricated stable photocrosslinkable PEC hydrogels using CHT and photocrosslinkable MeGG. This system is potentially useful for a variety of applications in regenerative medicine simply by changing the properties of the photocrosslinkable material and/or by changing the polymer charges, and therefore, the electrostatic interactions.

O110 (EI0138)

A GELATIN-BASED, CELL-FREE SCAFFOLD FOR THE TREATMENT OF ARTICULAR CARTILAGE DEFECTS – THE VALUE OF EARLY TISSUE REACTION IN AN ANIMAL MODEL

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Objectives: Scaffolds are used to treat articular cartilage defects. We developed an oriented gelatin-based scaffold material, which imitates the fibre orientation in the middle zone of articular cartilage. In an experimental animal study the early phase of integration and regeneration of the scaffold combined with microdrilling was analyzed and compared to microdrilling alone.
Methods: According to a standardized procedure, two punch defects were generated in the medial and lateral condylus of the knee in five goats and treated with microdrilling. After randomization, half of the defects were filled with a scaffold. After 3 months the defect sites were explanted and analyzed histologically by H&E and alcian blue staining.
Results: Both groups showed regeneration of cartilaginous tissue with a mild predominance of hyaline cartilage in the scaffold group. The scaffold group revealed good regeneration of the subchondral bone. In the control group, necroses, and sequestration of the subchondral bone were evident. Furthermore, the scaffold group showed a good integration and regeneration from the edges of the defect, but not the control group. The superficial zones of the defects were free of blood vessels in the scaffold group, but not in the control group.
Discussion: The blood vessel-free regeneration of hyaline cartilage was superior in the scaffold group compared to the control group. The pore orientation of the scaffolds might result in an optimized differentiation of inflowing cells and preferred formation of hyaline cartilage, since this orientation mimics the extracellular microenvironment of native cartilage. This could also be the reason for the lack of blood vessels in the scaffold group, since the scaffold-assisted differentiation into hyaline cartilage avoids the liberation of angiogenic factors.
Conclusions: This study shows the impact of early time points analyses for a better understanding of scaffold integration regarding microenvironmental effects.

O111 (EI0072)

SULFATION OF GLUCOSAMINOGLYCAN EFFECTS ON PROTEIN ADSORPTION AND CELL ADHESION

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Objectives: Protein- and cell-glucosaminoglycan (GAG) interactions have attracted great interests in the development of new therapeutics, for instance, tissue regeneration or cancer treatment. Most GAGs in nature possess sulfate groups, which contribute to multiple downstream signaling pathways. It has been reported that 6-O-sulfation of heparin is essential in FGF2 signal transduction, while 3-O-sulphation is required for anticoagulant activity. Here hyaluronic acid (HA), the only non-sulfated GAG existing in nature, has been studied as natural GAG and after sulfation. HA and sulfated HA surfaces were prepared through covalent bond between their oxidized derivatives and model surfaces, to study protein-GAG interaction and effects on cell-adhesion.
Methods: Chemical structures of oxidized HA (ox-HA) and sulfated HA (ox-HAS) were indentified by FT-IR. Surface properties, namely, wettability, charges, morphology and average roughness, were examined by different physical methods. The immobilization of oxidized GAG and the adsorption of aggrecan

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were monitored by surface plasma resonance (SPR). Adhesion of human fibroblast on the different surfaces was studied by microscopy analyzing overall morphology, number and spreading area of cells.
Results: FT-IR showed that the vicinal hydroxyl groups of HA and sulfated HA backbone were oxidized into dialdehydes, through which they could be immobilized on amino-silane modified substrata. The surface wettability and roughness increased after immobilization of oxidized molecules. In comparison with NH_2-modified surface, zeta-potentials of ox-HA and ox-HAS modified surfaces decreased to more negative values, which could be attributed to the presence of acidic dissociable surface groups. Compared to non-sulfated HA, the sulfated contributed to higher aggrecan adsorption and human fibroblasts adhesion.
Conclusions: Sulfation of HA has a significant effect on protein- and cell-GAG interactions. Covalent immobilization of oxidized GAGs seems to be useful to engineer cellular attching behaviour, which might pave the way to tissue enginnering.

Partial cardiac support in short- and long-term application - *SYMPOSIUM*

O112 (EI0117)
HEMODYNAMIC ANALYSIS OF EFFICACY OF PULSATILE PERFUSION DURING CPB WITH A NEW CENTRIFUGAL PUMP
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Objectives: New models of centrifugal pumps are claimed to have better hemodynamic performance in pulsatile perfusion during CPB. Few data are available for hemodynamic evaluation of these pumps in vivo, especially in high-risk groups as elderly patients. The study aims at comparing hemodynamic effects of pulsatile versus non-pulsatile perfusion using MEDOS DeltaStream-DP3 centrifugal pump in patients over 75 years old.
Methods: Forty patients with severe aortic stenosis (mean age 80.7±3.3, mean EuroScore 5.8±1.4) undergoing AVR from 1.01.2010 to 31.01.2010 were prospectively randomized into pulsatile (n=20pts) and non-pulsatile groups (n=20pts). Pressure and flow curves were recorded simultaneously from external flow-meters (TransonicHT110) and pressure monitor at 6 time points during CPB (at pre-oxygenator, post-oxygenator, aortic cannula and patients radial artery levels). Pulsatility was quantified in terms of Energy Equivalent Pressure(EEP) and Surplus Hemodynamic Energy(SHE). Hemodynamic indexes and clinical effects were monitored during 24 hours peri-operatively.
Results: Groups showed no difference in mean CPB time (p=0.98), cross-clamp time (p=0.95), mean perfusion flow (p=0.32) and pressure (p=0.16) values. In both groups the measured blood flow corresponded to the calculated one. Mean SHE generated at the outlet of the pump was 113.5±21.8 ergs/cm^3 with further progressive drop along the circuit until 5.3+1.9 ergs/cm^3 calculated in the patient (4.7% from initial level). The pulsatile group showed lower vascular resistance during CPB (p=0.035) and significant difference in SVR (p=0.04) and PVR (p=0.02) just after operation. Levels of SHE delivered to the patient correlated positively with urine output during CPB (R=0.34, p=0.041) and PVR after CPB (R=0.44, p=0.015). No differences between groups were found in pharmacologic support, transfusion rates, creatinine levels, respiratory indexes and intubation time. Longer ICU and hospital stay were related to severity of preoperative co-morbidities.
Conclusions: Pulsatile flow produced by MEDOS DeltaStream-DP3 centrifugal pump results in hemodynamic advantages and better tissue perfusion in high-risk patients.

O113 (EI0430)
HEMODYNAMICS OF A VALVELESS COUNTERPULSATION HEART ASSIST DEVICE: PARTICLE IMAGE VELOCIMETRY AND WALL-PARTICLE IMAGE VELOCIMETRY
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Objectives: The long-term application of counterpulsating devices (CPD) is limited by blood stagnation-induced thrombus formation. A new CPD with 32mL stroke volume was designed to prevent this cause of thrombus formation. Utilizing two measurement techniques, the blood washout behavior and wall shear stresses in the new design are obtained. A set of operating mode conditions, based on the time of membrane movement, were tested.
Methods: The time resolved flow in the disk-shaped lumen of the valveless CPD was investigated with Particle Image Velocimetry (PIV) and with wall-Particle Image Velocimetry (wall-PIV). The PIV study is focused on the central plane of the blood pump, where maximal velocities are anticipated. The wall-PIV investigations consider potential areas of blood stagnation on the housing of the blood pump.
Results: Flow investigations found that a tangentially designed CPD inlet port forms during a filling phase a strong, generally two-dimensional (2D) moving vortex fully filling the blood chamber. No regions of persistent blood stagnation or recirculation bubbles were observed. Shear stresses were moderate inside the blood chamber. The velocities in the lumen decay exponentially. Temporal vortex behavior was analyzed by its circulation frequencies. By comparison of wall shear rates and circulation frequencies the stagnation risk caused by different operation conditions was investigated.
Conclusions: The time resolved flow in the CPD lumen shows good washing characteristics with no stable areas of blood stagnation. By setting a threshold for the shear rate a maximal hold time can be identified.

O114 (EI0104)
MATHEMATICAL SIMULATION OF CLINICAL SCENARIOS DURING SUPPORT WITH CONTINUOUS FLOW LEFT VENTRICULAR ASSIST DEVICES (CF-LVAD)
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Objectives: Mathematical models can have important clinical diagnostic potential during mechanical circulatory support. In this research, we considered a mathematical model of the assisted heart and circulation with low complexity and established its potential to simulate multiple clinical scenarios of patients with cf-LVADs.
Methods: The heart was modelled with the one-fiber model concept which relates cardiac pump function with myofiber mechanics. The circulation was modelled by lumped parameter models. A cf-LVAD was modelled based on pump characteristics measured ex vivo. The model parameters were adapted to simulate clinical scenarios during cf-LVAD support related to heart failure, myocardial tissue remodelling, aortic valve competence and obstruction of the cf-LVAD.
Results: The simulation revealed that heart failure was characterized by increased ventricular dimensions, and decreased cardiac output and ejection fraction. The cf-LVAD led to decrease in dimensions and arterial pulsatility and caused permanent aortic valve closure. Improved contractility of the myocardium (cardiac recovery) led to improved arterial pulsatility and more aortic valve opening while, increase in myocardial tissue stiffness (atrophy) did not affect these parameters. Cf-LVAD flow increased with increase in aortic valve insufficiency while total cardiac output decreased. Aortic valve stenosis led to decreased cardiac output in the case of a pump stoppage. A large inflow or outflow obstruction area was necessary to cause a significant decrease in flow through the cf-LVAD.
Conclusions: In conclusion, we present a mathematical model which simulates the effect of heart failure, myocardial remodelling aortic valve competence and cf-LVAD dysfunction. Such model can become an appropriate prognostic tool for the development of novel therapeutic strategies for patients supported by cf-LVADs.

O115 (EI0393)
PREDICTING OXYGENATOR FAILURE DURING VA-ECMO: PERFORMANCE INDEXES
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Objectives: Prolonged extracoproeal support for cardiac assist (VA-ECMO) is associated with a progressive impairment of oxygenators (ML) performance. Once MLs became inefficient change-out (ChO) is mandatory and should be performed electively to decrease morbidity due to circulatory lack of assistance during ChO procedure. Up to now ML are changed according to clinical nonstandardized criteria. We analyzed effectiveness of variations in resistances to blood flow (BFR), O_2 transfer capability (TO_2), D-dimer and a combination of BFR-TO_2 (performance index, PI=BFR*TO_2) in predicting ChO need.
Methods: We rewieved 2008-2010 clinical data of all the patients treated with VA-ECMO in the cardiac ICU of San Gerardo University Hospital; ECMO technology was uniform (centrifugal pump Jostra Rotaflow, PLS oxygenator,

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Maquet, Germany). Exclusion criteria were: pre-existent disease affecting coagulation, fibrinolytic therapy, VA-ECMO duration <6 days. Decision for ChO was made according to clinical judgement. ROC analysis was performed on day-2 and day-1 data after setting the last day of ML that did not need ChO at the average day of ChO.
Results: 31 patients were enrolled, (54% males, 64±15 years old) accounting for 35 ML (10 ChO). ChO occurred on day 6.4±3.8. Best predictive parameters resulted PI (average±SD 993±541 and 1025±451 at baseline and ChO day respectively in CG) and the percent variation of D-dimer: AUC were respectively 0.80 (78% sensibility, 88% specificity, cut-off 1460) and 0.88 (88% sensibility, 87% specificity, cut-off 243%) at the day-1, and 0.66 (80% sensibility, 56% specificity, cut-off 1046) and 0.81 (89% sensibility, 75% specificity, cut-off 119%) at day-2. No correlation was found between D-dimer and PI variations.
Conclusions: An index combining TO_2 and BFR, and increase in D-dimer may predict oxygenator failure with high sensibility and specificity.

Artificial Organs – Practical Applications – *GENERAL SESSION*

O116 (EI0337)
AN INNOVATIVE SERUM-FREE CULTURE SYSTEM FOR EXPANSION AND OSTEOGENIC DIFFERENTIATION OF ADIPOSE AND AMNIOTIC-DERIVED STEM CELLS
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Objectives: Increase of life expectancy in the last decades resulted in more frequent aging problems, especially organ failure and tissue malfunctioning, which cause reduction of the Quality of Life. Present methodologies are still insufficient, therefore the ultimate solution may rely on the development of appropriate regenerative medicine strategies. Underlying these goals, there is the need for expansion of stem cells (SC), which are the primary players of tissue regeneration. Although several studies have reported the use of MSCs from adipose tissue, amniotic fluid (AF) and placenta in clinical settings, major hurdles for their clinical use are related to the need of animal supplements, and the small number of cells that can be isolated despite the large number of cells needed. Thus, our goal is to evaluate the proliferative and osteogenic potential of adipose- and AF-derived stem cells expanded in an enhanced serum-free culture system.
Methods: We isolated hAFSCs from the supernatant of day 6 cultures of amniotic fluid obtained from amniocentesis, and adipose stem cells (hASCs) from liposuctions. Cells were expanded in alpha-MEM supplemented with IGF-I and -II, bFGF and PDGF-BB, and compared to commercially available MesenCult®-XF. Cell expansion was evaluated by cell counts and DNA quantification. Expanded cells "stemness" and osteogenic potential were evaluated by flow cytometry and qRT-PCR.
Results: It was observed a progressive increase in cell number with the culturing time. Cell proliferation is favoured by the increase in initial cell density, demonstrating the importance of cell-cell interactions. This effect is more pronounced in ASCs cultures. Increasing the cell passage, we observed a loss of proliferative potential independently of serum-concentration in the culture medium, for the SC of both origins.
Conclusions: With this work we expect to contribute to the paving of the way to produce a culture system envisioning its use in regenerative medicine and broader clinical applications.

O117 (EI0275)
THE NEXT-GENERATION NANOCOMPOSITE MATERIALS FOR THE DEVELOPMENT OF TISSUE-ENGINEERED ORGANS AND TISSUES
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Objectives: Persisting organ shortages concurring with exponentially rising demands for donor tissues and organs have sparked a biotechnological race for the synthesis of artificial alternatives. Recently, fundamental advancements within the field of tissue engineering have furthered the potential for developing optimised bio-artificial substitutes. This comprises the growth of new tissue in a biological or synthetic scaffold within a bioreactor. In our laboratories, we have developed and patented a family of new generation nanocomposite polymers based on polyhedral oligomeric silsesqioxane (POSS) integrated poly(carbonate) urea-urethane (PCU) (non biodegradable) as well as the biodegradable pendant POSS integrated poly(caprolactone) urea-urethane (PCL) for the creation of 3-dimensional scaffolds for surgical applications.
Methods: Here, we present the characterization of our nanocomposite polymers including methods of fabrication, relevance of surface nanotopography in relation to biocompatibility and cell attachment, cellular integration and viability as well as their proliferative capacity. Polymers were fabricated using electrospinning as well as ultrasonic atomization spraying techniques. Utilising a tissue engineering approach, 3-dimensional polymeric scaffolds were created, characterized and integrated with various cell types including endothelial progenitor cells (EPCs) and adipose-derived stem cells (ADSCs).
Results: Integration of the POSS nanocages into the polymeric scaffolds conferred material biostability, anti-inflammatory and anti-thrombogenic properties and changed the surface nanotopography of the scaffold to create a more favourable extra-cellular environment for cells seeded onto it. EPCs and ADSCs were successfully grown on the nanocomposite scaffolds and showed viability as well as proliferative capacity.
Conclusions: There remains an unmet clinical need for effective scaffolds for tissue engineering biological substitutes. We have succeeded in developing new generation nanocomposite materials based on smart, bioactive, nanostructured materials to develop new types of tissue engineering scaffolds for the regeneration of tissues and organs.

O118 (EI0333)
INTRACELLULAR METHYLPREDNISOLONE RELEASE TO GLIAL CELLS USING AN ENGINEERED DENDRIMER NANOPARTICLE SYSTEM
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Objectives: SCI therapies do not present effective solutions due to the lack of efficiency of the drugs used. One possible approach to circumvent this could be the use of cell-specific intracellular drug delivery systems that could act on the neuroprotection and regeneration of the lesion. Thus, we are proposing the use of a dendrimer-based nanoparticle system composed of a polyamidoamine (PAMAM) core and grafted with carboxymethylchitosan (CMCht). These nanoparticles will be loaded with methylprednisolone (MP), an anti-inflammatory corticosteroid that would be carried to the site of lesion.
Methods: CMCht/PAMAM dendrimer nanoparticles (NPs) were synthesized and MP was incorporated. MP-loaded NPs were labeled with fluorescein isothiocianate (FITC) to evaluate internalization and intracellular trafficking. Particle size and zeta potential analysis were performed using the Zetasizer equipment. MP release profile was assessed by HPLC in two different buffer solutions (pH 5.0 and pH 7.4). Finally, glial and microglial cultures were established to evaluate the NPs behavior when in contact with these cells.
Results: MP-loaded NPs possess diameters around 109 nm and are stable at physiological pH. They do not affect glial cells viability or proliferation. Also, they were easily internalized by all CNS cell types reaching 100% internalization 24 hours after NPs addition. MP release profile revealed an initial burst within the first 24 hours followed by a sustained release for periods up to 14 days. The anti-inflammatory profile of these NPs was assessed in microglial cell cultures. The MP released from the NPs induced a significant decrease on microglial viability.
Conclusions: These results indicate that these dendrimer-based NPs have potential to be used as modulators of the inflammatory events in SCI sites. Additionally, they are excellent intracellular delivery carriers, entering the cells at high rates and releasing the incorporated drug within its cytoplasmic compartment, and allowing its action to be carried out.

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O119 (EI0106)

EFFECTS OF UNFRACTIONED HEPARIN AND LOW-MOLECULAR-WEIGHT HEPARIN ON OSTEOPROTEGERIN AND RANKL PLASMA LEVELS IN HEMODIALYSIS PATIENTS

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Objectives: This randomized crossover study investigated the effects of unfractioned heparin (UFH) and low-molecular-weight heparin (LMWH) on intra- and post-dialytic blood levels of osteoprotegerin (OPG), receptor activator of nuclear factor kappa B ligand (RANKL) and inflammatory cytokines in dialysis patients.
Methods: We selected 40 patients on hemodialysis for 12 months. UFH or LMWH was randomly assigned and maintained for 1 month, and then each patient was switched to the other form of heparin. In the mid-week session, we determined anti-Xa activity, OPG, RANKL, IL-1β, IL-6 and TNF-α values before heparin administration and after 15 min, 4, 8 and 24 h (T0, T1, T2, T3 and T4, respectively).
Results: A highly significant (P<0.001) increase in anti-Xa activity was detected at T1, regardless of the type of heparin (UFH or LMWH), as confirmed in the comparison of T0 vs T1 using one-way ANOVA. Moreover, with both heparins, significant differences were found at T1 vs T2 (both P<0.001) and at T2 vs T3 (P=0.0003 with UFH; P<0.001 with LMWH). Conversely, the difference in anti-Xa activity at T3 vs T4 was still significant with UFH (P=0.0186) but not with LMWH. Anti-Xa activity at T4 vs T0 was not significantly different either with UFH or LMWH, indicating that 24h after heparin infusion, it returned back to pre-infusion values. Changes in OPG levels over time, regardless of the type of heparin, showed an increase in circulating OPG with a zenith at T1, and a return back to the baseline levels within the 24 hours post-infusion. Significant differences in OPG blood levels were shown at T0 vs T1 with both UFH (P=0.0112) and LMWH (P=0.0288).
Conclusions: These results suggest that heparin-regulated cyclic increases of OPG might play a role in vascular pathology of hemodialysis patients.

O120 (EI0083)

ACCELERATED REMOVAL OF TNF WITHIN A HEMOADSORPTION DEVICE USED TO TREAT SEPSIS

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Objectives: Sepsis, systemic inflammation due to infection, is characterized by high concentrations of inflammatory cytokines in the circulating blood. Extracorporeal blood purification using biocompatible, microporous sorbent beads has been shown by us to decrease circulating cytokine levels and increase survival time in septic rats. The objective of this study was to develop novel techniques to accelerate capture of TNF, a large pro-inflammatory cytokine, within the hemoadsorption device. We propose surface modified sorbent beads capable of dissociating trimeric TNF into monomeric form, thereby accelerating removal of TNF from the circulating blood.
Methods: TNF in horse serum was incubated with 10% DMSO to promote deoligomerization of trimeric TNF. Size exclusion chromatography was used to determine effects of DMSO incubation on TNF molecular size. Effects of TNF deoligomerization on capture were evaluated using an *in vitro* recirculation loop. TNF (~1ng/mL) was spiked into 8mL horse serum, and recirculated through a column packed with 1.5g sorbent beads. Aliquots were periodically sampled from the reservoir, and TNF concentration was quantified using ELISA.
Results: Native TNF eluted as a 34kD oligomer using size exclusion chromatography. Incubation with 10% DMSO dissociated the TNF oligomer and resulted in elution of a 10kD monomeric form. TNF capture from serum within the device was significantly accelerated after DMSO incubation, compared to native TNF capture (95% removal vs. 64% removal after 4 hours, respectively).
Conclusions: TNF capture within the hemoadsorption device is slow due to the large size of trimeric TNF. TNF removal was significantly accelerated by dissociating oligomeric TNF, thereby allowing fast diffusion of monomeric TNF into the sorbent pores, and enhancing overall removal of bioactive TNF. We are developing surface modified sorbent beads to locally dissociate TNF within the device, as a novel method for increasing TNF removal rates while retaining an optimal sorbent pore size for albumin exclusion.

O121 (EI0240)

EPOETIN-α/DARBEPOETIN-α SWITCH IN HEMODIALYSIS PATIENTS: TRIAL TO EVALUATE THE TREATMENT EFFICACY IN ACHIEVING HEMOGLOBIN TARGET LEVELS AND OPTIMIZATION OF COST/EFFICACY RATIO

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Objectives: Recombinant human erythropoietins (rHuEPOs) as epoetin-α and epoetin-β are effective in the treatment of renal anaemia. Darbepoetin-α differs from rHuEPOs for longer serum half-life and greater erythropoietic activity. Aim of the study was to compare the efficacy of the switch and associated costs from epoetin-α to darbepoetin-α for the treatment of anaemia in hemodialysis using a new dose conversion ratio (DCR).
Methods: 78 patients were treated. The visits were defined as follows: T1) start of epoetin-α therapy; T2) 6 months after T1, switch to darbepoetin-α; T3) 1 month after T2, evaluations after the switch; T4) 6 months from T3, final evaluations. The DCR generally used to change epoetin-α to darbepoetin-α is based on the protein mass equivalence between molecules (200:1). According to our routine clinical practice of erythropoiesis stimulating agents (ESA) management, the initial DCR was set up to 254:1.
Results: At T2 epoetin-α mean dose was 13,717.95IU/week (equivalent to a mean dose of 53.91μg/week of darbepoetin-α), whereas the mean darbepoetin-α dose at T3 was markedly reduced (p<0.05) to 50.26μg/week and 44.36μg/week at T4. The mean Hb values measured at T1, T2 and T3 were 11.05g/dL, 11.19g/dL and 11.4g/dL, displaying a progressive increase from T1 to T3 and a significant increase between the concentrations observed at T2 and T3 (p<0.01). An increase in the mean Hb corresponds to a decrease of the mean weekly dose of administered darbepoetin-α (p<0.05). The proportions of patients reaching Hb target ≥11 and ≥12g/dL were higher after darbepoetin-α (65.7%vs29.3%) compared to epoetin-α (57.4%vs25.2%). DCR was 335.37.
Conclusions: Both ESAs are effective in renal anaemia. However, while epoetin-α group showed a modest increase of hemoglobin levels, darbepoetin treated patients had a better improvement. Our results suggest that, in order to maintain stable hemoglobin levels, the use of a DCR superior to 250:1 is required. Clinical benefits apart, darbepoietin-α can provide economical benefits.

NON-DESTRUCTIVE TECHNIQUES TO MONITOR 3D IN VITRO TISSUE ENGINEERING CONSTRUCTS - *SYMPOSIUM*

K20 (EI0095)

OPTICAL TECHNIQUES FOR MONITORING 3D TISSUE CONSTRUCTS

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Objectives: Optical techniques offer potential for functional imaging over a wide range of cell scales from sub-cellular through to whole organ. An overview of optical techniques that are currently being applied to monitoring of 3D tissue constructs will be provided. The focus will then fall on (i) the use of a combined optics plus ultrasound system for monitoring tissue growth in scaffolds and (ii) the application of machine vision methods to monitoring cell aggregation in rotary cell culture systems.
Methods: An overview of non-linear microscopy and optical coherence tomography will be provided. An ultrasound modulated optical tomography system has been constructed to image tissue constructs in 3 dimensions, which involves laser illumination and an ultrasound transducer. Light passing through the focus becomes modulated at the ultrasound frequency allowing the light to be 'tagged' and the effects of light scattering to be reduced. A machine vision and imaging processing system has been developed to automatically monitor cell aggregation.
Results: Key recent results from non-linear microscopy and optical coherence tomography will be highlighted. Images of absorbing and fluorescent targets embedded in tissue scaffolds (gels and foamed scaffolds) will be presented. Images of the cell aggregation process in a rotary cell culture system and properties extracted from the images (e.g. number of aggregates v time) will be shown.
Conclusions: Optical techniques can provide functional imaging and monitoring over a wide range of sizes, from the sub-cellular to whole organs. This offers the potential for both better research tools and in industrial scale up of artificial organs.

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O122 (EI0389)

NON-DESTRUCTIVE QUALITY CONTROL FOR ISLET TRANSPLANTATION USING RAMAN SPECTROSCOPY

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Objectives: Type 1 diabetes patients with poorly controllable glucose levels can be treated by intrahepatic transplantation of donor islets of Langerhans. Before islets are transplanted into the patient, their quality needs to be assessed. Current quality control requires fixation and labeling and does not allow time-lapse studies on the same tissue. In this study we explore the feasibility of using Raman spectroscopy to perform functional studies on pancreatic islets and to monitor their quality over time.
Methods: Raman measurements were performed using a home-built confocal Raman spectrometer. A krypton ion laser emitting at 647.1 nm and a 40x air objective were used to excite the sample, and the Raman scattered photons were collected and dispersed on an air-cooled electron-multiplying charge-coupled device.
Results: We first used Raman spectroscopy to measure purified insulin and glucagon, the two main hormones produced by pancreatic islets. Raman bands at 520 and 640 cm^{-1} can be assigned to cysteine and tyrosine, amino acids that are present in insulin. Tryptophan, one of the building blocks of glucagon, causes specific bands at 759 and 1552 cm^{-1}. These bands can be used as markers for the identification of beta and alpha cells in islet preparations. We subsequently measured human islets and compared their spectral characteristics to those of insulin and glucagon. Tryptophan-specific Raman bands were observed in the islets spectrum, suggestive for the presence of glucagon-producing alpha cells. Bands suggestive for insulin were not observed in the average islet spectrum, possibly because insulin is a weaker Raman scatterer. High-resolution local measurements on individual islet cells are currently performed to identify the presence of insulin-vesicles inside these cells.
Conclusions: Our data provides the first steps towards a non-destructive and label-free method to study pancreatic islet quality before transplantation in type 1 diabetes patients.

O123 (EI0039)

THE STUDY OF OPTICAL PROPERTIES AND PROTEOGLYCAN CONTENT OF NATIVE AND TISSUE ENGINEERING TENDONS BY PS-OCT

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Objectives: Tendons are load-bearing collagenous tissues consisting mainly of type I collagen and various proteoglycans (PGs). It is widely accepted that highly orientated collagen fibers in tendons play a critical role for transferring tensile stress and demonstrate birefringence optical properties. Although PG's are the essential components of the tendon extracellular matrix, the influence of proteoglycans on its optical properties is yet to be fully elucidated. On the other hand, it is not fully clarified what is the key factor which regulates tendon optical properties during its regeneration. The objective of this study is using a nondestructive optical image technique to study the effect of PG content on collagen fibril organization during PG extraction in terms of birefringence alteration; also identify the essential parameters which can alter tendon's spatial organization manifesting as birefringence alteration.
Methods: Fresh chicken leg tendons were dissected and used within 48 hours of dissection. The total proteoglycan was extracted by 4M/2M guanidine hydrochloride. The acellular engineered tendons made from polylactic acid nanofiber and collagen hydrogel were fabricated and the density of nanofiber and collagen hydrogel were varied. A bench-top fibre based time-domain polarization sensitive optical coherence tomography (PS-OCT) system was used to acquire the cross-section birefringence images during the PG extraction up to two hours. The PS-OCT images of acellular tendons were taken at different fabrication conditions.
Results and Discussion: Extraction of GAG resulted in distortion of birefringence bands in native tendons. Higher concentration and longer extraction time led to destruct the birefringence bands rapidly and completely. Using low collagen density hydrogel did not generate acellular tendons with birefringence bands no matter the presence of nanofiber or not.
Conclusions: The non-destructive imaging technique, PS-OCT, is a reliable and simple monitoring tool for studying spatial structure of highly organized tendon tissue for quality control or regeneration regulation.

O124 (EI0295)

NOVEL APPROACH FOR IN VIVO NON-INVASIVE VASCULAR GRAFT COMPLIANCE MEASUREMENT

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Objectives: Compliance mismatch between a vascular prosthesis and the adjacent native artery has been related to neointimal hyperplasia leading to graft failure. A significant amount of research is currently being led in the field of degradable vascular grafts and proper characterization of mechanical properties in vitro is customary. However, mechanical properties of these structures in vivo may significantly evolve due to tissue remodeling and material degradation, but these changes are generally poorly monitored and described. The aim of this study is to report a new non-invasive method of in vivo vascular graft compliance measurement in the rat model.
Methods: Five male Sprague Dawley rats (275g) received an infrarenal abdominal aorta replacement with a poly(ε-caprolactone) (PCL) electrospun prosthesis. High-resolution ultrasonography (VEVO 770, VisualSonics Inc., CAN) was performed to assess systolic and diastolic internal diameters of implants and adjacent abdominal aortas. Non-invasive arterial systolic and diastolic blood pressure measurements (CODA Monitor, Kent Scientific Corp., USA) were simultaneously carried out. This data was used to calculate in vivo compliance.
Results: The average compliance of implanted PCL grafts was 9.4±0.5%/100mmHg. Proximal and distal native aortas had an average compliance of 26.38±4.2%/100mmHg and 21.5±4.5%/100mmHg, respectively. The compliance of the native aorta was significantly higher than PCL grafts (p=0.0014) revealing a compliance mismatch.
Conclusions: A reliable measurement of native and prosthetic vascular compliance in vivo is feasible in small animal models. This new method can provide time-related insight on compliance changes of biodegradable vascular grafts and lead to better design of next generation vascular grafts.

O125 (EI0004)

DEVELOPMENT OF AN IMPLATABLE SMALL CAMERA FOR ANGIOGENESIS

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Objectives: Angiogenesis is essential for successful tissue-engineered artificial organs and regenerative medicine. However, the mechanisms and conditions required for angiogenesis have not been disclosed yet. In this study, we monitored growth of blood vessels in vivo to study the angiogenesis mechanism.
Methods: A miniaturized video camera system integrated with a scaffold for blood vessels and tissue induction that is implantable into an animal body was developed. A polyglycolic acid sheet of 0.3 mm thickness was used as a scaffold. The camera was implanted with the scaffold, and we observed angiogenesis into the scaffold.
Results: We observed tissue induction to the scaffold. It was started from the second week and it covered the entire area by the tissue about 10 weeks. Vigorous angiogenesis was observed at the front region of tissue induction resulting in dense distribution of capillary vessels and red blood cells.

Stent and Vascular Prosthesis – *GENERAL SESSION*

O126 (EI0408)

***IN VIVO* EVALUATION OF ELECTROSPUN, BIODEGRADABLE AND NON-DEGRADABLE ELASTOMERIC VASCULAR GRAFTS**

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Objectives: Biodegradable polymer scaffolds for tissue engineering applications have been the focus of intensive research during the last years. Polyurethanes (PU) are ideal candidates for vascular substitutes due to their excellent mechanical

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properties. In this study we evaluated the in vivo behavior of degradable and non-degradable, electrospun polyurethane grafts in a rat model.
Methods: Biodegradable (aliphatic PU, n=8) and non-degradable (Pellethan 2563-80A, n=8) mesh grafts were fabricated by electrospinning (void fraction 80%, fiber diameter 0.8-1.5 μm, inner diameter 1.5 mm, wall thickness 100μm, length 15 mm). E-ptfe grafts (n=8, inner diameter 1.5, wall thickness 100μm, 25 μm IND, length 15 mm) were used as controls. Grafts were implanted into the aorta of 24 rats and analyzed after 6 months by biomechanical analysis, immunohistochemistry, scanning and transmission electron microscopy and morphometric techniques.
Results: All grafts showed no signs of thrombus formation and aneurysmal dilatation. Both PU mesh grafts showed significantly increased transmural cellular ingrowth and complete endothelial coverage. More than 50% of the original wall of the aliphatic PU grafts was replaced by vascular specific tissue. Neointima formation was increased in eptfe and in aliphatic PU grafts. Tensile tests revealed a loss of strength of 12% for the non-degradable PU graft and of 45% for the degradable PU conduit.
Conclusions: Both PU mesh grafts have the potential to attract vascular specific cells. It has to be evaluated in long-term studies if balanced tissue remodeling may compensate the loss of mechanical strength of the biodegradable implants.

O127 (EI0041)
IN-BODY TISSUE-ENGINEERED AND COMPLETELY AUTOLOGOUS AORTIC VALVED CONDUIT (BIOVALVE) IN A GOAT MODEL
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Objectives: Autologous valved conduits without any artificial materials (BIOVALVE) were developed, using simple, safe, and economical in-body tissue engineering. In this study, the potential of the BIOVALVE as an aortic valve was investigated in a goat model.
Methods: BIOVALVEs were prepared by 2-month embedding of the molds, assembled using 2 types of plastic rods, in the subcutaneous spaces of goats. After removing the molds, BIOVALVEs with 3 leaflets and sinus of Valsalva similar to those of native aortic valves consisted of perfectly autologous connective tissues. The BIOVALVEs were implanted as aortic valves using the apico-aortic bypass method for 2 months.
Results: Postoperative echocardiography and angiography showed smooth movement of the leaflets with little regurgitation under systemic circulation. Histological examination after 2 months showed that α-SMA–positive cells appeared significantly with rich angiogenesis in the conduit and expanded toward the tip of the leaflet. At the sinus portions, marked elastic fibers were formed. The luminal surface was covered with thin pseudointima. **Conclusions:** Completely autologous BIOVALVEs with robust and elastic characteristics satisfied the higher requirements of systemic circulation in goats for 2 months.

O128 (EI0189)
DEVELOPMENT OF TAILOR-MADE SEMILUNAR TRANSCATHETER HEART VALVES
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Objectives: Transcatheter heart valve implantation is a young and innovative technology offering a novel form of treatment to patients previously classified as "no-option patients", due to comorbidities preventing open heart surgery associated with surgical valve replacement. Initial clinical results of transcatheter aortic and pulmonary valve replacement demonstrate promising results but also reveal the need for improvement. Factors which require technological development are repositionability and anchoring of prostheses to prevent device migration. Since suturing the valve into place is not possible in a transcatheter implantation, anchoring must be achieved through optimal anatomical fit.
Methods: The diversity of pathological morphologies of the left and right ventricular outflow tract in patients indicated for transcatheter heart valve surgery leads to poor anatomical fit, paravalvular leakage or prosthesis migration. This study presents a new strategy for designing and manufacturing transcatheter heart valves in a system referred to as the "custom assembly kit". In this system, the prosthesis is composed of up to three components, one base valve element, having three leaflets, as well as proximal and distal anchoring elements. These three components can be assembled according to the anatomicy of a specific patient anatomy and provide optimized anchoring to prevent valve migration.
Results: CAD designs and early prototypes of the concept demonstrate its feasibility. Excellent anatomical fit of the device is achieved by offering different diameters and geometries for the anchoring elements to connect to the base valve element. The assembled device offers the best possible prosthesis for a given pathological valve and outflow tract morphology. The design and development of this device are performed in close collaboration between biomedical engineers and cardiologists to ensure optimal mechanical durability, longevity and anatomical fit.
Conclusions: This system represents the next step in transcatheter heart valve technology and will be further developed to form a marketable device.

O129 (EI0163)
THE SORIN FREEDOM STENTLESS PERICARDIAL VALVE: A VALID BENCHMARK FOR CURRENT PERCUTANEOUS DEVICES
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Objectives: Trans-catheter aortic valve implantation (TAVI) has emerged as an alternative to aortic valve replacement (AVR) for severe aortic stenosis. The majority of TAVI systems currently available incorporate pericardial stentless bioprostheses, with only short follow-up data available. Therefore information on long-term results of AVR with a stentless pericardial valve, such as the Sorin Freedom (SF), represents a valid benchmark. In this study we report the clinical and hemodynamic performance of SF with a 10-year follow-up.
Methods: From January 2000 to December 2004, 78 patients, mean age 5.6±5.8 years, underwent AVR with SF. Sixteen (20.5%) were in NYHA class IV, 18 (23.1%) in III, 44 (56.4%) in class I or II. Mean ejection fraction (LVEF) was 58.11±11.16%. Echocardiographic evaluation was performed at 3, 12 months and yearly thereafter assessing effective orifice area (EOA), gradients (PG) and regression of left ventricular mass index (LVMi).
Results: There was 1 operative death (1.2%). A total of 77 patients were discharged and followed for total follow-up of 5602 months (mean 70±25 months). There were 24 late deaths with an actuarial survival of 56±8.8% at 10 years. Three patients were reoperated, with a freedom from reoperation of 95±3% at 10 years, because of structural deterioration, endocarditis and dilatation of sinotubular junction, respectively. At last clinical control 47 patients (90%) were in NYHA class I or II and 5 patients (9%) were in NYHA class III. Mean EOA varied from 1.8±0.8cm^2 for valve size21 to 2.3±0.6 cm^2 for size27 and mean PG varied from 22±9 mmHg for valve size21 to 13±4 mm Hg for size27. LVMi decreased from 182.9±39.6gm/m^2 to 142.1±42.6gm/m^2 ($p<0.001$).
Conclusions: SF stentless bioprosthesis has provided good results in terms of valve durability and freedom from valve-related complications with excellent hemodynamic performance at 10-year follow-up. These data represent important reference point against which performance of current TAVI systems must be compared.

O130 (EI0249)
A NOVEL APPROACH FOR THE DEVELOPMENT OF POLYURETHANE VALVE LEAFLETS
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Objectives: Modern heart valve prostheses are either mechanical disk valves or biological prostheses with porcine or bovine pericardial leaflets. Mechanical heart valves demonstrate good durability, but require lifelong anticoagulation due to thrombosis and hemolysis. Biological prostheses do not require anticoagulation, but are prone to calcification and degeneration. The use of polyurethane as a leaflet material allows the combination of durable synthetic materials with the flow characteristics of tricuspid biological prostheses. To make use of these advantages, a reproducible manufacturing process is required. In this study a novel development approach for polyurethane valve leaflets, manufactured from polyurethane tubes, is presented.
Methods: In order to manufacture polyurethane heart valves with high durability, low residual stresses are required. This is initially achieved by molding polymer films from medical grade polyurethane and winding these to tubes. The edges of the films are connected using solvent. The manufactured tubes were then mounted on polymer stents with edges representing the form of the leaflet commissures of natural tricuspid valves. In order to improve valve kinematics, the leaflets were clamped at the commissure tips and joined. The resulting heart

valve prostheses were tested in an accelerated wear tester. First endurance tests were performed with distilled water, at a testing frequency of 10 Hz and physiological pressures. With this setup, 6 samples were tested to failure.
Results: All tube valve samples showed good closing behavior and pressure response. The tested valves endured up to 12 Mio. cycles before failing by tearing along the leaflet edges. Leaflet fracture resulted in pressure loss without fragmentation.
Conclusions: Artificial heart valve leaflets manufactured from polyurethane tubes give an encouraging perspective for future heart valve prostheses. Further investigation is focused on seamlessly manufactured tubes, optimized pre-forming of the valves and different polyurethane material grades.

O131 (EI0020)
THE BIOSTENT – NOVEL CONCEPT OF A VIABLE STENT STRUCTURE
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Objectives: The percutaneous stent angioplasty of peripheral vessels is well established as clinical routine. Unfortunately the patency rates of small-caliber grafts (< 6mm) are still unsatisfying, especially in the lower limb region. The aim of a novel BioStent concept is to overcome vessel occlusion due to both a total exclusion of the atherosclerotic plaques from the blood stream and an intact, functional active endothelial cell layer. The proposed concept bases on the combination of a self-expanding stent technology with the principles of vascular tissue engineering: the moulding process of vascular grafts, based on a fibrin gel scaffold, allows the complete integration of a self-expanding stent structure within the tissue-engineered vessel. With this completely new principle the major causes of restenosis (1 the foreign body reaction, (2 the cell proliferation with ingrowth in the lumen and (3 acute thrombosis by hemo-incompatibility will be prevented. The reason is the total exclusion of the atherosclerotic section from the blood stream and the coating of the neolumen with a functional endothelium, including the antithrombotic function of the endothelial cells.
Methods: Small-caliber (6mm) BioStents were made by combining a self-expanding nitinol stent with a thin fibrin-based tissue-engineered blood vessel. The remodelling of the fibrin scaffold with mature autologous proteins was tested by histological analyses. A confluent endothelial cell monolayer lining the luminal surface of the Biostent was shown by scanning electron microscopy.
Results: A thin coverage of about 200µm completely wrapping the stent structure was achieved. With the scanning electron microscopy a total lining with endothelial cells could be observed.
Conclusions: The present feasibility study shows the successful combination of a self-expanding nitinol-stent with a fibrin-based tissue-engineered blood vessel. Further investigations like integrity of the luminal endothelial cell layer and in vivo studies are necessary to proof a percutaneous applicability and will be implemented soon.

Dialysis Techniques Access – *GENERAL SESSION*

O132 (EI0021)
FROM THE DIALYSIS OUTCOMES AND PRACTICE PATTERNS STUDY (DOPPS): THE IMPACT OF SEASONS ON CENTRAL VENOUS CATHETER RELATED SEPTICEMIA
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Objectives: While central venous catheter-related sepsis (CRS) contributes to adverse health outcomes in hemodialysis (HD) patients, unexplored risk factors remain, such as season of the year and central venous catheter (CVC) dressing protocols.
Methods: Data of 8412 HD patients in 12 countries from DOPPS I and II (1996-2004) were analyzed. CRS was defined as septicemia during or within 15 days after HD CVC use. Catheter time at risk (n=1,754,293 days) and CRS were assigned to 1 of 4 seasons in each country. CRS relative rates (RRs) by season and the association of facility CVC dressing protocols with hazard ratio (HR) of CRS were determined by Poisson and Cox regression, respectively.
Results: Overall CRS rate was 1.2/month or 0.41/(1000 CVC days) (0.47 in North-America), ranging from 0.34 during "fall" and "winter", over 0.38 in "spring", to 0.49/(1000 CVC days) during "summer". CRS varied by month with a maximum CRS in August [0.57/(1000 CVC days)], and an adjusted RR for "summer" of 1.42 [95% CI (1.09-1.87)] compared to reference 1 for "winter". With respect to the dressing protocol, CRS was lower using betadine [adjusted HR=0.81, 95% CI (0.65,0.996)], or chlorhexidine [HR=0.81 (0.59,1.11)] compared to alcohol, with attenuated HRs (0.87-1.06) for combined cleansing agents. Furthermore, CVC infection rates varied by personnel type who typically inspects the CVC access site and/or changes dressing protocol: nephrologist [HR=0.64 (0.45,0.92)], technician [HR=1.47, (0.98,2.2)] compared with nurse as reference.
Conclusions: The higher CRS rate in summer may be due to higher heat, humidity, and perspiration, potentially facilitating bacterial growth and compromising protective measures. Extra vigilance by staff may reduce CRS in this high-risk season. Betadine and chlorhexidine may be more effective than other cleansing agents.

O133 (EI0024)
HEMODYNAMIC IMPACT OF HELICAL DESIGNED GRAFTS: COMPARISON WITH STRAIGHT CONVENTIONAL GRAFTS
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Objectives: In case of low-quality vessels, artificial arterio-venous grafts (AVGs) offer the best long-term vascular access for hemodialysis. AVGs, however, deal with complications such as thrombosis and stenosis, the latter finding its origin in intimal hyperplasia, which is mainly located in regions of low Time Average Wall Shear Stress (TAWSS), high Oscillatory Shear Index (OSI) and high Relative Residence Time (RRT), mainly at the venous anastomosis or in the draining vein. The hypothesis that a helically designed AVG reduces intimal hyperplasia formation through the reduction of these unfavorable hemodynamic conditions is studied.
Methods: Four 3D CFD-models of an AVG (6mm diameter) between an artery (4mm diameter) and a vein (6mm diameter) in loop configuration, were studied: one conventional straight graft design, and three helical designs with a pitch of 105mm, 70mm and 35mm, respectively. All models were meshed with a hexahedral, structured and conformal grid. A physiological pulsatile blood flow was used as inflow (average 600mL/min), while the distal artery and vein had an outflow of 5%, and the proximal vein 90%.
Results: The area of TAWSS below 1 Pa lowered by 12%, 0.8% and 60% for progressively more helicity in the design, whereas the area of OSI above 0.1 decreased by 7%, 61% and 80% and the area with an RRT above 1 Pa-1 with 12%, 13% and 55%. All helical designs thus reduced the area of low TAWSS, high OSI and high RRT. The design with pitch 105 mm already lowered the area with high OSI, while only the strong helical design (pitch 35 mm) lowered the areas with low TAWSS and high RRT.
Conclusions: The areas with unfavorable hemodynamic conditions at the upper wall of the venous anastomosis can be lowered using a strong helical design but not fully eliminated.

O134 (EI0031)
ALTERNATIVE DIALYSIS VASCULAR ACCESS IN COMPARISON TO STANDARD RADIAL-CEPHALIC ARTERIOVENOUS FISTULA
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Objectives: Arteriovenous fistula (AVF) between the radial artery and the cephalic vein at the wrist is the most preferred configuration of dialysis vascular access. However, in patients with inadequate cephalic veins that are not suitable for constructing standard AVFs, we need to create alternative AVFs at different sites or using non-native materials. In this study, we evaluated the characteristics and AVF survivals in the patients with standard and alternative AVFs.
Methods: We constructed a standard radial-cephalic AVF at the non-dominant wrist when the cephalic vein was sufficiently large (approximately 2 mm or larger in diameter). Meanwhile, when the vein was not available, we constructed an alternative AVF at a different site or using a prosthesis. In both groups,

medical records including age, gender, co-morbid conditions and a history of surgical procedures were retrospectively analyzed. In addition, primary and assisted survival of AVFs which were created in the first recent year were also determined.
Results: In 305 first-time vascular access surgeries performed from 2006 to 2010, we constructed 207 standard AVFs (68%) and 98 alternative ones (32%) such as antecubital AVFs. The patients with alternative AVFs, compared with standard ones, were significantly older (71.8 y.o. vs 67.7 y.o.) and more female-gender (39% vs 27%). A history of previous surgery seemed to significantly reduce the likelihood to create standard AVFs (odds ratio 2.55, 95%CI 1.41-4.62, p=0.002). Although alternative AVFs required more frequent radiological intervention (30%), their assisted one-year survival was 79%, which was as high as that of standard AVFs (94%).
Conclusions: It seemed that a history of previous surgery was a risk factor for successful standard AVF construction, suggesting that peri-operative venipunctures potentially affected the vascular availability. It also seemed that alternative AVFs, once created at different sites with available veins, are comparably useful as dialysis accesses.

O135 (EI0035)
SUSPECTED CATHETER-RELATED BLOODSTREAM INFECTION – NONTUNNELED VS TUNNELED HEMODIALYSIS CATHETERS
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Objectives: Complication of central venous catheterization for hemodialysis are catheter thrombosis and catheter-related bloodstream infection (CRBI). We report rates of bacteremia experienced with all types of central venous catheters (CVC). We used femoral and subclavian catheters as non-tunneled CVC (NTCVC) and tunneled CVC (TCVC)-femoral, subclavian and jugular catheters.
Methods: In a prospective study we looked at the outcome of a group of 620 patients (pts) with acute and chronic renal failure treating with hemodialysis via a 738 CVC, during a 3-year period. Catheters were placed by nephrologists in a femoral, internal jugular and subclavian vein, and episodes of catheter infection were recorded. Each catheter was followed individually until it was removed or until the end of the study.
Results: In total, 539/738 (74.9%) of procedures were insertions of NTCVC, whilst 181/738 (25.1%), were insertions of TCVC. Of the NTCVC insertion, 501/738 (69.6%) were inserted into femoral vein, with 38/738 (5.3%) in subclavian vein. In a group of TCVC – 103 were tunneled femoral (56.9%), 41 – tunnelled jugular (22.6%) and 37 subclavian (20.5%) catheters. A total of 44 576 catheter days were accumulated over the study period during which time were 42 cases of CRBI (infective rate 2.57 episodes/1000 catheter days). Rates of CRBI were 2.77 episodes/1000 cath. days in the TCVC group; 3.5 per 1000 cath days in NTCVC. Multivariant analysis demonstrated hazard ratios (HR) for the development of CRBI in pts dialysing with subclavian NTCVCs of 2.9 [95% confidence interval (CI) 1.5-4.8, p<0.001], and femoral NTCVC of 3.9 [95% CI 2.1-7.4, p<0.001]. The infection and malfunction free survival time was with statistically significant difference between NTCVC and TCVC.
Conclusions: We concluded that recognizing and knowing the risk factors can prevent complications of tunneled hemodialysis catheters. TCVC insertions have an association with lower complication rates than NTCVC insertions

O136 (EI0056)
A NEW METHOD OF MONITORING BLOOD PRESSURE AND CARDIAC OUTPUT IN CHRONIC DIALYSIS PATIENTS
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Objectives: Hypotension and circulatory failure are common problems during dialysis sessions. These problems occur often suddenly without any early signs. Short-term non-invasive blood pressure measurement (method of Riva-Rocci) is often not tolerated by the patient so that a decreasing blood pressure is not detectable early enough. A new circulation monitoring system would be helpful.
Methods: We established a method for the measurement of the blood pressure in the arteriovenous shunt of chronic dialysis patients during discontinued blood flow in the vascular access. A measurement and analysis system evaluates the pressure fistula values, which correlate with systemic blood pressure and analyse further parameters (slope of the increasing mean arterial pressure after stopping the blood flow in the vascular access, waveform of every single pulse). We investigated 10 stable chronic dialysis patients, suffering from chronic heart failure and hypotension during dialysis sessions. The systemic blood pressure was measured with an automated device (Dinamap, GE Helthcare). Measured values of both systems were compared every 15 minutes.
Results: Measured values of both methods differed, but the trend during a dialysis session was the same. The blood pressure in the shunt and especially the slope of the blood pressure after stopping the blood flow in the vascular access depend on shunt characteristics (age, location, maximum blood flow, prevalence of stenosis). The maximum blood flow in the shunt depends on systemic circulation. The new measurement procedure is well tolerated by the patient, altough it is performed in a very short term. The sensitivity of the detection of decreasing blood pressure values is improved. Early signs of circulation failure are detectable, especially if the evaluation of fistula blood flow characteristics is obtained.
Conclusions: The new measurement is a save method for detecting circulatory failure during dialysis sessions in patients with high risk of circulation failure.

O137 (EI0207)
TWO SINGLE-LUMEN NON-CUFFED CATHETERS IN THE JUGULAR VEIN AS A HEMODIALYSIS VASCULAR ACCESS FOR MORE THAN 100 DAYS
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Objectives: Two single lumen catheters in the same jugular vein have been used as a preferred vascular access in our hemodialysis (HD) patients since 2006. With this study we wanted to retrospectively analyze clinical outcome of such a vascular access and reasons for catheter removal in those patients where catheters were not removed for at least 100 days.
Methods: In 121 adult patients (53 females and 68 males), as a vascular access two single lumen pre-curved 8F catheters (Medcomp, Harleysville, USA) inserted in the same jugular vein were used between January 2009 and April 2010. 30% solution of a 3Na-citrate was used as a locking solution, and 2% Mupirocin ointment was applied on the exit site routinely. Analysis was performed 6 months after the end of inclusion period.
Results: In 20 patients (10 females and 10 males, age 70.2±11.3 years) catheters were not removed or exchanged for at least 100 days. At the time of analysis in 4 patients the catheters were still functional. 3 patients died with functional catheters. The longest duration of such a vascular access was 387 days and this patient died with the functioning and not infected vascular access. In 6 patients the catheters were removed because AV fistulas were constructed. In 6 patients exchanges over guide wires were performed for the correction of displaced functional catheters, and in 1 patient the catheters were removed at day 184 because of an infection.
Conclusions: Two non-cuffed single lumen pre-curved 8F catheters inserted in the same jugular vein, locked with 30% citrate, seem to be a safe long lasting vascular access for some HD patients, but further prospective studies are needed to evaluate clinical outcome and complications of such a vascular access.

SCAFFOLDS FOR TE VIA ELECTROSPINNING-STRUCTURES AND BIOMATERIALS - *SYMPOSIUM*

K21 (EI0445)
DESIGNING ELECTROSPUN SCAFFOLDS FOR TISSUE ENGINEERING
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Objectives: Electrospinning is a facile technique for production of polymer fibres with diameters in sub-micron to micron range. Resulting scaffold microstructure closely resembles the extracellular matrix and can be enhanced further by incorporating various signalling molecules such as proteins, growth factors etc. in fibres. This is achieved by modifying the standard electrospinning process *via* blend, emulsion and co-axial electrospinning.
Methods: Water-soluble polymers such as PEO were functionalized by blend electrospinning by mixing the protein solutions with polymer solution prior to electrospinning. Stability of the fibres was achieved through a cross-linking step post-electrospinning. Water-insoluble polymers were functionalized using emulsion and co-axial electrospinning. For the former, protein solutions were emulsified in water-immiscible polymer solutions with a surfactant, while for the latter, a specially designed concentric nozzle setup was employed. Fibres were collected on different collector geometries to obtain different scaffold macrostructures.

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Results: The process was influenced by different parameters such as concentration, voltage, flow rate etc. Proteins were incorporated in fibres and released via diffusion. For blend electrospinning, burst release of proteins was observed along with protein denaturation. Both problems were solved by using the modified electrospinning procedures (emulsion or co-axial electrospinning). Different 3D constructs such as heart valve conduits and tubes for vascular grafts were produced.
Conclusions: The electrospinning process could be easily modified to allow functionalization of fibres with proteins. Of the 3 different methods, blend electrospinning was the least favourable, since it caused protein denaturation. On the other hand, protein activity was retained using emulsion or co-axial electrospinning for fibre functionalisation. 3D macrostructure heart valves or blood vessels could be replicated by using different collector geometries.
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O138 (EI0063)
MEMBRANE BIOENGINEERING STRATEGIES TOWARDS THE DEVELOPMENT OF A BIOLOGICAL KIDNEY SUPPORT DEVICE
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Objectives: Despite hemodialysis therapy, many end-stage renal disease patients have uremic complications, resulting in a poor quality of life. Uremia is caused by the retention of a large group of molecules that are not adequately cleared by hemodialysis. Attempts to improve this situation by convection or high-flux dialysis membranes were not very successful. In our study, we develop living membranes for the removal of protein bound uremic toxins by renal epithelial cells. In our concept, we use a composite membrane having two layers: the first layer allows protein bound toxins to reach the cells but simultaneously blocks immune cells, immunoglobulins and complement factors. The second layer made of bioactive electrospun mesh favors the adhesion of renal epithelial cells, which will assist on the toxins removal.
Methods: For the first layer, Flat and hollow fiber (HF) membranes were either prepared in house by phase inversion or purchased. For the second layer, polycaprolactone (PCL)-based fiber mesh was produced by electrospinning and integrated with the first membrane layer. The morphology of the produced membranes was investigated via SEM, whereas the transport properties were studied using water and protein model solutions at selected pressures.
Results: For the protein permeable membrane, polysulfone-based homemade or commercial membranes were studied. The tested membranes have high water permeance (1500 – 2200 L/hr/m^2/bar), high albumin sieving coefficient (SC, higher than 0.8) and rather low immunoglobulin IgG transport (SC < 0.3). The cytocompatible fiber mesh was obtained by electrospinning of polycaprolactone (PCL) and integrated onto the protein permeable membrane by either direct electrospinning or lamination.
Conclusions: Direct electrospinning compromises water and BSA transport through the composite whereas the transport remained unaffected when the PCL membrane is produced separately and later laminated onto the membrane. Besides flat composites, the preparation and characterization of capillaries is in progress, too.

O139 (EI0220)
ASSESSMENT OF ALIPHATIC THERMOPLASTIC POLYURETHANES FOR NARROW DIAMETER VASCULAR GRAFTS
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Objectives: Thermoplastic polyurethanes (TPUs) find use in demanding medical applications such as catheters and pace maker casings. In addition to good resistance to thrombosis, TPUs have better mechanical compliance than traditional vascular graft materials (Dacron or PTFE) and are actively researched for use as small-bore (< 5 mm) grafts. Most commercially available TPUs are based on aromatic isocyanates which offer excellent mechanical properties and stability however raise concerns over toxicity of decomposition products. Such concerns are compounded if the graft is intended to be fully or partially degradable, two approaches in vascular grafting which seek to assist rather than replace native tissue growth.
Methods: TPUs based on aliphatic isocyanates, oligomeric diols and different ester containing chain extenders have been synthesized. Polymers were fabricated into forms for mechanical testing and simulated biodegradation. Toxicity was assessed both in vitro with HUVECs and in vivo by subcutaneous implantation. Aliphatic TPUs were also electrospun into nanofibrous conduits and implanted as aortic interponates into Sprague rats.
Results: Mechanically, aliphatic TPUs tend to be softer than aromatic TPUs although the modulus can be adjusted with appropriate chain extenders. Procedures for electrospinning tubes from TPUs are also amenable to aliphatic derivatives. Fiber diameter, mesh density, and wall thickness are all well controlled. HUVECs seeded on aliphatic TPUs attach and grow well. Live/dead assays maintain that cells stay viable in the presence of polymer and its degradation products. Initial results from implantation show no complications.
Conclusions: Aliphatic TPUs with cleavable chain extenders have flexible mechanical properties and provide potential advantages in degradable or partially degradable vascular grafts. Tuning of modulus and rate of degradation is possible by blending or copolymerization. Electrospinning TPUs provides nanofibrous tubes appropriate for vascular grafting.

O140 (EI0064)
INTEGRATION OF HOLLOW FIBER MEMBRANES IMPROVES NUTRIENT SUPPLY IN 3D TISSUE CONSTRUCTS
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Objectives: Despite the great progress in tissue engineering, development of clinically relevant size tissues with complex architecture remains a great challenge mostly due to limitations of nutrient and oxygen delivery to the cells. This study focuses on the development and utilization of a new perfusion culture system using hollow fiber membranes to provide adequate nutrient delivery to the cells within large three-dimensional (3D) scaffolds.
Methods: Three-dimensional scaffolds were created by (i) rolling pre-seeded electrospun sheets around porous hollow fiber (HF) membranes and (ii) by integration of HF within free form fabricated (FFF) scaffolds. The culture of pre-myoblast (C2C12) cells under static and dynamic conditions on these scaffolds was investigated in a dedicated bioreactor. In fact, dynamic medium perfusion occurred via the HF lumen and around the 3D scaffolds. Various parameters such as fiber transport properties, fiber positioning within a scaffold, and medium flow conditions were optimized. The scaffolds were analyzed using scanning electron microscopy (SEM), histology and DNA assay.
Results: The hollow fibers act as additional source of nutrients and oxygen to the cells by providing medium through the porous walls in a controlled manner at low shear stress. The SEM analysis and histology shows clearly that only integration of fibers achieves homogenous cell distribution within the scaffolds whereas the total DNA assay shows quantitatively high cell proliferation within the scaffolds. In the case of the electrospun multilayer scaffolds, cell migration occurs within the construct (shown using pre-labeled C2C12 cells) illustrating the potential of using our concept for developing more complex tissues.
Conclusions: This study demonstrated the proof of concept of using polymeric hollow fibers as artificial capillaries for nutrient delivery to rather large 3D tissue constructs and could provide a basis for a new culture methodology for developing such constructs.

O141 (EI0355)
ELECTROSPUN FISH GELATIN: EFFECT OF CROSS-LINKING METHODS ON CELL GROWTH
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Objectives: This study was performed in order to produce electrospun fish gelatin scaffolds and evaluate the effect of different cross-linking methods on cell growth and proliferation.

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Methods: Fish gelatin (from fish skin – Sigma G7041) is soluble in cold water. In this work, it was dissolved in: distilled water, at a concentration of 40 wt% (solution S1); acetic acid:distilled water (90:10 in wt) at concentrations of 25 wt% (S2) and 18 wt% (S3). The cross-linking methods used were: electrospun mats obtained from S1 and S2 were exposed to glutaraldehyde vapor or dehydratated at low pressure and high temperature; 2% genipin was added to S3 and the solution was electrospun after 6 days. The mats obtained from S3 were then immersed in an ethanol:water (90:10 in wt) solution with 2% genipin in order to increase the cross-linking degree. Scanning electron microscopy was used to characterize the morphology of the scaffolds and effectiveness of the cross-linking procedure. 3T3 fibroblasts were cultured on the fiber mats and confocal microscopy images were used to observe the morphology of the cells by immuno-staining with phaloidin and ToPro3.
Results: Electrospun gelatin nanofibers obtained from S1 and S2 were defect-free with regular fiber diameters while those from S3 presented varying diameters and some fiber bonding. After cross-linking, some structural degradation occurs, in particular with glutaraldehyde and genipin. All nanofibrous mats became insoluble after cross-linking. Confocal images revealed good adhesion and proliferation of the cells cultured on all scaffolds, with those cross-linked with genipin showing the best results.
Conclusions: Solutions of fish gelatin were successfully electrospun. The scaffolds were cross-linked using three different methods. The cell culture study showed that cross-linked gelatin from fish skin is a promising scaffold for soft tissue engineering.

Drug Delivery Systems – *GENERAL SESSION*

O142 (EI0155)
TAT PROTEIN TRANSDUCTION INTO ISOLATED PERFUSED HEART AND *IN VIVO* DURING CARDIOPULMONARY BYPASS
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Objectives: Linkage of the 11-amino-acid transduction domain of HIV TAT to a heterologous protein allows the protein to be transduced readily across cellular membranes into cells. The aim of the study was to investigate the effectiveness of delivery of peptides into cardiomyocites *ex vivo* in isolated perfused heart and *in vivo* during cardiopulmonary bypass (CPB) using the newly developed TAT protein transduction system.
Methods: TAT undecapeptide fused with green florescent protein (TAT-GFP) was infused *ex vivo* in isolated perfused heart (Langendorff) model for 60 minutes. Secondarily, TAT-GFP was infused *in vivo* in a rat model of CPB lasting for 60 minutes. These procedures were followed by a 15-minute washout period, which was followed by perfusion-fixation with formaline.
Results: Confocal microscopy revealed homogeneous distribution of TAT-GFB in myocardium sliced from apex to base of the heart. TAT-GFP was localized in >90% of cardiomyocytes both *ex vivo* and *in vivo*.
Conclusions: These results demonstrate that TAT protein transduction may be a promising tool for myocardial protection in cardiac surgery.

O143 (EI0264)
GROWTH FACTOR-DELIVERING DEVICES FOR TISSUE ENGINEERING APPLICATIONS
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Objectives: The rationale for the use of growth factors in Tissue Engineering applications is given by their ability to stimulate the controlled proliferation and differentiation of the seeded cells in the scaffolds. They should also enhance the migration, proliferation, and differentiation of cells from the edges of the treated defect. We analysed three different approaches for integration of a potential growth factor prostaglandin E_2 (PGE_2).
Methods: The first approach utilizes an emulsion-based route to synthesize polymeric (PLGA) microspheres with incorporated PGE_2. These microspheres were used in a second approach to establish a three-dimensional scaffold system by distributing PGE_2-PLGA-microspheres in a gelatinous suspension followed by freeze-drying and moderate chemical cross-linking. The third system used the development of a PLGA scaffold with direct integration of the biosignal. All PGE_2 values were given as mean values after triple analyses via mass spectrometry assays.
Results: The first approach showed a kinetic release of biologically active PGE_2 with a burst release of PGE_2 over the first two days and continued release over 8 days. The distribution of growth factor-loaded microspheres allows a gradient of growth factors due to the spatial distribution of the microspheres. The incorporation efficacy of the fluid-foamed system was higher than different preparations of microspheres. This third approach demonstrated that the direct incorporation of PGE_2 into a polymer foam is possible without extensive loss of the growth factor.
Conclusions: Growth factors act in a dose-dependent manner and via receptors on the target cells. Therefore, the kinetics of growth factor release from delivering devices should be adapted to the situation of the microenvironment. The amount of growth factor in the tissue or the cell-scaffold construct must reach an optimum for its biological action in balance, which is one major feature for the biomaterial design.

O144 (EI0175)
AMPHIPHILIC BLOCK COPOLYMERS: SYNTHESIS AND CHARACTERIZATION OF MULTIFUNCTIONAL MICELLES FOR TARGETED DRUG DELIVERY BY RAFT POLYMERIZATION
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Objectives: Reversible addition-fragmentation chain transfer (RAFT) polymerization belongs to the controlled/living radical polymerization methods and is highly efficient in the preparation of well-defined drug and gene delivery vesicles.
Methods: Via RAFT Polymerization different amphiphilic block copolymers consisting of poly(N- acryloyl morpholine) or poly(N-isopropylacrylamide) and poly[2-hydroxyethyl methacrylate-block-poly(ε-caprolactone)] have been prepared. Two different trithiocarbonate RAFT agents have been used and AIBN acted as initiator. As fluorescence marker in cell imaging Fluorescein methacrylate was copolymerized in the hydrophobic block. Micelles were formed by dialysis and their hydrodynamic diameter was characterized by dynamic light scattering (DLS).
Results: We successfully prepared well-defined amphiphilic block copolymers with low polydispersity indices by RAFT polymerization. They are composed of poly(N-acryloyl morpholine) and poly(N-isopropylacrylamide) as hydrophilic part and biodegradable poly[2-hydroxyethyl methacrylate-poly(ε-caprolactone)] in the hydrophobic part. The ability of these block copolymers to self assemble into micelles in an aqueous surrounding was determined by DLS. The uptake of the micelles into living cells was shown by fluorescence imaging. Further modification of the block copolymers is possible due to succinimide units in the polymer backbone.
Conclusions: Based on biodegradable amphiphilic block copolymers we designed new smart drug carriers, which can assemble into micelles. Because of succinimide groups in the hydrophobic part these block copolymers are able to bind drugs with an amino functionality. The fluorescein moiety included in the polymer backbone allows to monitor the cellular drug uptake by fluorescence imaging. Enzymatic degradation studies of the micelles are currently ongoing.

O145 (EI0044)
DUAL PRO-ANGIOGENIC THERAPY BASED ON THE RELEASE OF VEGF165 AND ENDOTHELIAL CELLS DERIVED FROM UMBILICAL CORD STEM CELLS IN A HYBRID NETWORK
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Objectives: We report a new approach to integrate in the same 3D hydrogel scaffold a growth factor delivery system and a matrix able to support the adhesion and remodeling of endothelial cells derived from hematopoietic stem cells.
Methods: Dextran with variable degree of oxidation (DexOx) was conjugated with $VEGF_{165}$ and the conjugates characterized by SDS-PAGE and circular dichroism. The bioactivity of the conjugates was evaluated in $CD34^+$-derived endothelial cells (ECs) by their potential to trigger Ca^{2+} uptake and to activate ERK and Akt signaling pathways. Radiolabelling and ELISA were used to

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monitor VEGF release from dexOx-VEGF hydrogels. DexOx-VEGF microgels were encapsulated in fibrin hydrogels, loaded with ECs, and the cellular viability was evaluated up to 6 days. During this period, the organization and gene expression of ECs was evaluated by microscopy and qRT-PCR.
Results: VEGF was immobilized into DexOx having variable degree of oxidation (DO). Circular dichroism results show preservation of the 3D structure of immobilized VEGF. In addition, immobilized VEGF activates the intracellular concentration of Ca^{2+}, ERK and Akt signaling pathways. Dextran hydrogels containing immobilized VEGF have variable release rates depending on their crosslinking density. The release of VEGF within the hybrid gel induced a cord-like organization of the endothelial cells. This effect was only observed for constructs containing low oxidized dextran hydrogels. In addition, no visible network formation was observed in low DO constructs without VEGF, indicating that the formation of cord-like structures was mediated by VEGF. Finally, constructs that release faster VEGF present cells with a downregulation in the expression of VEGF gene and an upregulation of MMP-2 gene.
Conclusions: The hybrid construct allows the independent tailoring of the drug release system from the cell-bearing matrix and was shown to modulate gene expression and three-dimensional endothelial cell organization.

O146 (EI0033)
EFFECT OF LIPOSOME-ENCAPSULATED HEMOGLOBIN AFTER TRANSIENT COCHLEAR ISCHEMIA AND REPERFUSION IN THE GERBIL
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Objectives: Liposome-encapsulated hemoglobin (LEH), an artificial oxygen carrier, has been proved to be protective when administered before cochlear ischemia and reperfusion. In the current study, LEH was tested after transient cochlear ischemia and reperfusion as a therapeutic model of sudden deafness.
Methods: Mongolian gerbils, devoid of circle of Willis, were randomly assigned to receive 2 mL/kg of LEH with high O_2 affinity ($P_{50}O_2$=17mmHg) or saline (each n=6) one hour after 15-min occlusion of the bilateral vertebral arteries to induce transient cochlear ischemia. Sequential changes in hearing were assessed by auditory brain response at 1, 4, and 7 days after ischemia/reperfusion, when the animals were sacrificed for pathological studies.
Results: LEH was significantly more effective than saline in suppressing hearing loss over a wide auditory range at 8kHz, 16kHz, and 32 kHz in contrast to the saline treatment which was associated with significant hearing loss in each auditory range (P<0.05). Although the degrees and differences in hearing loss were gradually reduced as time passed after ischemia/reperfusion 1, 4, and 7 days, when the inner hair cell loss was significantly suppressed in LEH-treated animals (P<0.05).
Conclusions: The results suggest that post-ischemic treatment with LEH (2 mL/kg) with high O_2 affinity ($P_{50}O_2$=17mmHg) is effective in mitigating hearing loss (function) and inner hair cell loss (morphology) following transient cochlear ischemia and reperfusion as an experimental model of sudden deafness.

O147 (EI0253)
MORPHOLOGY OF POLYMER MATRICES CONTAINING RISPERIDONE
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Objectives: Nowadays innovation in the therapy of schizophrenia is achieved by applying novel atypical antipsychotic agents and also by novel drug formulations. Long-acting risperidone in the form of injection is a relatively recent solution acting during two weeks. The acting agent in the commercial product is micro-encapsulated in poly(lactide-co-glycolide) and formed in microspheres. We propose implantable and biodegradable solid form that allows prolonged action and explantation in comparison with the commercial product.
Methods: Risperidone-loaded matrices (10 wt-% of drug substance) were obtained from high molecular poly(L-lactide-co-glycolide) 85:15 by solution casting method. Morphological properties of polymeric matrices were evaluated by atomic force microscopy (AFM) during 9 months of degradation. Degradation processes of polymeric matrices with diameter of 10 mm were carried out in phosphate buffered saline (pH 7.4) at the temperature of 37°C under constant agitation conditions.
Results: During degradation processes the changes in morphological and morphometric features between non-degraded and degraded matrices were revealed. Non-degraded matrix showed solid structure with single pores. Incubation of matrices in phosphate buffered saline under the constant agitation conditions resulted in the appearance of significant porosity. Moreover, elongation of degradation period influenced the widening of pores' diameter and their deformation.
Conclusions: AFM study showed differences between risperidone-loaded matrix before and after 9 months of degradation, thus confirming the progression of the degradation processes. Determination of morphological and morphometric changes during the degradation process are important for designing biodegradable and implantable matrices releasing risperidone.

"Approval procedures for medical devices: facts, figures and basic rules seen from different continental perspectives – Artificial Organs and Society: Recent trends in Japan"

O148 (EI0277)
CURRENT STATUS OF INDUSTRY-ACADEMIA COLLABORATION ACTIVITIES IN JAPAN AND PRACTICE OF THE "NATIONAL CEREBRAL & CARDIOVASCULAR CENTER"
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Objectives: To develop new medical devices such as ventricular assist devices, which will be used in clinical practice, it can be considered that industry-academia collaboration had better to be conducted to lead by medical research institute. Medical research institutes in Japan had not generally conducted industry-academia collaboration activities organizationally, whereas industry-academia collaborations have become active in accompanied by shifted to independent administrative agency in the universities. The objective of this study is to clarify the current status of industry-academia collaboration in Japan including National cerebral & CardioVascular Center (NCVC), owing to promote technology commercialization on medical device development.
Methods: The present activities of industry-academia collaboration of NCVC was compared with that of universities which were surveyed by the Center of the Ministry of Education, Culture, Sports, Science and Technology of Japan. To compare the activities of industry-academia collaboration, the number of joint research contracts and patent royalty income were selected as indicators.
Results and Conclusions: The number of joint research contracts of universities in Japan during the last year (within the top 10) was about 300-1000 and patent royalty income was 340-1,100 thousand U.S. dollars. On the other hand, a department of intellectual asset management was established with the independent administrative agency shift of NCVC on April 2010, and promoted industry-academia collaboration activities. Under this new organization, NCVC entered into 52 joint research contracts and patent royalty income was 70 thousand U.S. dollars during the last year. NCVC has only about 200 researchers and medical doctors, whereas these universities have about 2,000-4,000 faculty and researchers, and hence these universities hold about 10-20 times manpower of NCVC. Considering manpower, our performance of joint research and patent royalty income is equal to within the top 10 universities. These suggest that NCVC can be propelled industry-academia collaboration effectively.

O149 (EI0146)
WIDE ACCEPTANCE FOR DOMESTIC MEDICAL DEVICES IN JAPAN
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Objectives: As for medical device business, harmful rumors often cause withdrawals of existing companies or entry barriers for new companies. Many Japanese manufacturing companies, especially small and medium enterprises, hesitate to enter into medical device business due to the fear for harmful rumors although they can provide high quality technologies. The objective of this study is to investigate the public attitudes toward the medical device field in Japan.
Methods: We designed an internet-based questionnaire providing basic information on current status of the medical device field and assessing the public

opinion on the issue; necessity, self-sufficient rate, approval, review periods, recalls, information, corporate images of the companies entering into medical device business from other business fields and attitudes toward Japanese medical device companies. The survey included a nationally representative sample of 5,155 adults excluding health care workers. The study period was from March 8, 2011 to March 9, 2011.

Results: Among the respondents, 94% recognized the growing necessity of medical devices; 83% required self-sufficient rate improvement of medical devices. As for regulation and safety, 27% poorly understood that medical devices were approved by a government organization; 63% expected shorter review periods; 90% realized that recalls contributed to the safety. Of the respondents, 80% expected to be provided with technical information. Concerning medical device companies, 63% considered that corporate image of the companies which entered into medical device business from other fields would improve; 99% supported for the domestic medical device companies.

Conclusions: The study revealed the wide acceptance for domestic medical devices in Japan. Japanese highly recommended contributions of domestic companies to medical device development. The public attitudes may motivate Japanese manufacturing companies to enter into medical device business.

O150 (EI0364)

ARTIFICIAL ORGANS IN COMPLEX EMERGENSIES OF EARTHQUAKE, TSUNAMI, AND NUCLEAR POWER PLANT ACCIDENT

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Objectives: Magnitude 9.0 earthquake and over 30 meter Tsunami attacked the Tohoku area on March 11, 2011. Furthermore, steam explosion attacked Fukushima nuclear power plant and radiation exposure was observed in Tohoku area. Details concerning artificial organs in Tohoku area will be presented in this session.

Methods: All systems in hospitals in Miyagi prefecture downed. Dialysis patients gathered to the Tohoku University Hospital, which have first priority in life-line in Sendai city. HD patients were transported to other national Hospitals by helicopter. Monitor, respirator and ventricular support systems were smoothly recovered after electric power supply.

Results: Dead or alive was decided in border of the Tsunami area. Patients with artificial organs were almost OK in Tohoku University Hpspital. Water supply was damaged at the City level, so it became the disturbance of HD. A lot of HD patients were transported by helicopter. The number of helicopters was too small in Sendai.

Conclusions: Repeated discussion must be performed for the emergency situation of the artificial organs.