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**CITRATE ANTICOAGULATION**

**O1 (172)**

**CITRATE ANTICOAGULATION FOR HEMODIALYSIS**

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Citrate has many characteristics of the ideal anticoagulant for hemodialysis. Its anticoagulant effect is excellent and is limited to the dialysis circuit. In addition to inhibiting coagulation, it reduces platelet deposition on the dialyzer membrane. By chelating calcium and magnesium, citrate reduces some of the effects of blood interaction with the dialyzer membrane, which are calcium and magnesium dependent, thus improving biocompatibility of dialysis circuit. Using citrate, we avoid heparin-induced thrombocytopenia. Citrate is easily dialyzable (molecular weight of trisodium citrate is 294 Da). Its removal during high-flux dialysis is 83%. The cost of citrate is low. The major issue preventing wider use of citrate is the complexity of citrate anticoagulation protocols.

Since 1993 we have been using 4% trisodium citrate for hemodialysis, plasma exchange and continuous procedures, prepared by our hospital pharmacy, which also prepares calcium chloride solution (1 M). Calcium-free dialysate is used, with potassium concentrations of 2, 3 or 4 mmol/L. Sodium and bicarbonate concentration are reduced on hemodialysis monitor. In 2009 we have performed 3074 dialysis procedures (8.2% of a total of 37,344 hemodialysis procedures). The majority, 60% (1837/3074) of citrate procedures were performed in chronic hemodialysis patients, the rest of them, 1237/3074 (40%) in acute patients, mostly in intensive care units. Some of our patients are treated by chronic citrate anticoagulation.

Besides standard hemodialysis we have successfully used citrate for single-needle hemodialysis, predilutional on-line hemofiltration and continuous procedures. The use of calcium-containing dialyzer 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 standard, calcium-containing infusate (1.25 mmol/L) and successful regional citrate anticoagulation.

Despite its complexity, citrate anticoagulation is safe if performed by trained nurses and doctors and offers so many advantages compared to other anticoagulation methods that we can expect an increase in the use of citrate in future.

**O2 (174)**

**CITRATE AS A LOCKING SOLUTION FOR HEMODIALYSIS CATHETERS**

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After decades of using heparin in various concentrations for catheters locking, citrate is gradually replacing it in clinical practice. Its advantages are avoidance of systemic anticoagulation, antimicrobial activity, low cost and biofilm reduction. According to our best knowledge, we were among the first who reported the use of citrate instead of heparin as a locking solution for hemodialysis catheters (1994). Our motivation was avoidance of systemic anticoagulation. We started to use 4% citrate, with more than 10,000 catheters locked with this solution in the years after. The majority of catheters were non-cuffed, femoral and jugular, used as temporary or long-term vascular access. In the minority of patients we have used 4% citrate for locking of silastic, cuffed single-lumen catheters. In 2002 Weijmer et al reported on antimicrobial effect of citrate, the significant advantage in addition to avoidance of systemic heparinization. After the randomized clinical study of Weijmer et al was published (comparing 30% citrate with heparin), we have switched to 30% citrate (from 4%). The main reason was better antimicrobial activity of more concentrated citrate. The question of optimal citrate concentration for catheter locking still remains unanswered. Higher concentrations have better antimicrobial activity, but they can cause temporary local hypocalcaemia or thrombosis at catheter tips (especially at side holes) and embolization. According to our experience and more than 10,000 catheters inserted annually, the majority of them non-cuffed and all locked with 30% citrate in the last years, including catheters for critically ill patients, and with more than 10,000 of catheters locked with 4% citrate before, we believe that concentrations of more than 30% are not necessary or advisable. It is also possible that locking solution should be chosen depending on the type of the catheter. Which citrate concentration is optimal for particular catheter type requires further study. We believe that citrate locking, in addition to catheter design and nursing care, enabled the use of non-cuffed ( temporary) catheters for long-term use, as we have been doing at our center for years.

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**O3 (66)**

**CHRONIC CITRATE ANTICOAGULATION**

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In maintenance hemodialysis regional citrate anticoagulation is used in patients which are at increased risk of bleeding. Citrate anticoagulation is invaluable especially in the setting of single-needle hemodialysis, where possibilities for efficient anticoagulation are very limited and citrate provides excellent anticoagulation limited to the extracorporeal circuit. In the majority of citrate anticoagulation protocols for (double-needle) hemodialysis calcium-free dialysate is used. The use of standard, calcium-containing dialysate was also described, which is more practical and possibly increases safety, by reducing the possibility of accidental hypocalcaemia due to technical error. We have compared the two protocols in randomized fashion and have shown that citrate anticoagulation using 1.25 mmol/L calcium dialysate results in significantly worse anticoagulation compared to calcium-free dialysate, in spite of more than 35% higher citrate dose. Furthermore, in some exceptional cases long-term (e.g. > 3 months) citrate anticoagulation is needed. The main indications would be heparin-induced thrombocytopenia, severe anemia due to persistent blood loss (e.g. from gastro-intestinal tract) or other persistent inclination to bleeding.

We present our series of 8 patients on long-term citrate anticoagulation (up to 60 months). The citrate anticoagulation protocol was simplified, the number of blood samples reduced and calcium supplementation was mainly modified by the attending nurse to maintain ionized calcium within target range. Questions deserving further attention in citrate anticoagulation are: first, optimal target values for arterial ionized calcium (should it be kept within normal range or slightly lower), especially with regard to stimulation of parathormone secretion and total calcium balance, and second, optimal circuit ionized calcium values.

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**O4 (211)**

**EXTENDED CITRATE HIGH-CUT-OFF HEMODIALYSIS FOR THE TREATMENT OF MYELOMA PATIENTS WITH DIALYSIS-DEPENDENT RENAL FAILURE**

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Objectives: Rationale for extended hemodialysis using high cut-off membrane (HCO-HD) is removal of large quantities of free light chains (FLC) in patients with myeloma associated kidney disease. We report two cases of multiple myeloma with dialysis-dependent renal failure that were treated intensively with chemotherapy and extended citrate high-cut-off hemodialysis.

Methods: Patient No.1 was a 74-year-old male with lambda FLC type of myeloma and cast nephropathy on renal biopsy. Patient No. 2 was a 63-year-old male, who presented with kappa type of myeloma and acute renal failure. Both patients received chemotherapy and hemodialysis with Gambro high-cut-off Theralite 2100 membrane which was performed 8 hours daily until successful reduction in serum FLC concentration was achieved. Patient No. 1 was dialyzed with bicarbonate hemodialysis but for patient No. 2 hemodiafiltration method was chosen in attempt to facilitate FLC removal. Anticoagulation with 4% trisodium citrate was used. Serum FLC levels were measured by immunoassay.

Results: Patient No. 1 received 18 bicarbonate HCO-HD sessions in 20 days and patient No. 2 received 9 HCO-hemodiafiltration sessions in 12 days. Citrate anticoagulation was effective and well tolerated. A successful reduction in serum FLC concentration was achieved. Patient No. 1 was dialyzed with bicarbonate hemodialysis but for patient No. 2 hemodiafiltration method was chosen in attempt to facilitate FLC removal. Anticoagulation with 4% trisodium citrate was used. Serum FLC levels were measured by immunoassay.

Conclusions: Two myeloma patients with dialysis-dependent renal failure undergoing aggressive regimen of high cut-off hemodialysis and hemodiafiltration were treated successfully in our center. We believe that early initiation of intensive HCO-HD may contribute to improvement of renal function in such patients.
OXYGENATION

O5 (268)
A NOVEL CONCEPT FOR HOLLOW FIBERS APPLIED TO OXYGENATORS:
SILICONE HOLLOW SPHERE FIBERS (HSSF)
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Objectives: Conventional hollow fibers (HF) like Polypropylene (PP) are microporous and have been exclusively used for commercial production of capillary membrane oxygenators (CMO) for the last three decades. The reason behind this wide spreading lies on its convenient gas exchange potential, although it is still far from the ideal demands. Apart from that, hazards such as plasma leakage, foam formation, and brittleness elevate the risk of blood trauma hindering long-term application of CMO. Attempts to overcome such technical difficulties were concluded in developing plasma resistant fibers, or through various silicone coating of capillaries, or even applying solid silicone fibers, yet not showing complete elimination of incompatibilities. Here, we introduce a novel type of pure diffusive silicone capillaries with walls embedding micro spheres: by enclosure of these micro spheres a high gas exchange performance is established, in addition to adequate stability, flexibility, and haemocompatibility of the silicone made biomaterial.

Methods: Further developed HSSF (manufactured by Raumedic® AG, Germany) with an inner diameter of 200µm and a wall thickness of 100µm with 40% ratio of embedded air spheres to impose the optimal compromise between structural stability and gas exchange efficacy. Small scaled oxygenator-modules for very low flow rates using HSSF as well as conventional HF as control modules were constructed for in vitro validation in an experimental circuit. The total surface area amounted to 0.03 m² with a priming volume of 4.2 mL. The modules were investigated with distilled water, and further with blood as the liquid and pure oxygen for the gas phase with a 1:1 flow ratio.

Results: The mean outlet pO2 of HSSF amounts to 460 mmHg for water and oxygen for the gas phase with a 1:1 flow ratio.

Objectives: Current oxygenation systems with an additional blood pump can cause several adverse reactions due to their large extrinsic surface contact area and high filling volumes. First tests with silicone tubes embedded in the fiber bundle of an oxygenator show an increase of gas exchange efficiency. Additionally, the extracorporeal priming volume can be reduced by using these tubes as a blood pump.

Methods: Oxygenator fibers woven into mats were used to manufacture cylindrical fiber bundles. Silicone tubes where embedded into these bundles. A pulsatile blood flow through the oxygenator can be generated by collapsing and expanding the silicone tubes due to air pressure pulses. Therefore, the additional volume of a pump can be saved. Moreover, the blood flow inside the fiber bundle can be improved by adequate positioning of the pumping tubes.

Results: In this study, fiber bundles with the same geometry with integrated silicone tubes were compared to bundles without tubes. The gas exchange inside the bundles with embedded tubes was 2 – 3 times higher irrespective of incoming flow (pulsatile or constant). In further experiments, the practicality of using the pulsating silicone tubes inside the hollow fiber bundle of the oxygenator as a pump could be confirmed. Oxygenator modules with high and low shunt flows have been compared. It was shown that the pulsating tubes within the bundle increased the gas exchange efficiency in an oxygenator with high shunt flows. While pumping with the integrated pulsating tubes, the gas exchange was 2 to 3 times higher than an external generated constant flow.

Conclusions: One positive achievement of the embedded silicone tubes is the increase of gas exchange efficiency of an oxygenator. By using the pulsating silicone tubes to generate a blood flow, there is no additional pump needed. This reduces the extrinsic surface contact area as well as the filling volume and would potentially expand the range of applications for oxygenators.

O6 (93)
TESTS OF THE ADDED RESISTANCE METHOD ON A VIRTUAL RESPIRATORY SYSTEM
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Objectives: Obstructive lung diseases, the fourth case of death, are usually related to increased airways resistance (RAW). Forced expiratory volume in one second (FEV1) is a good index of the obstruction but its dependence on the obstruction may be masked by other factors. RAW has to be therefore determined directly in many cases and a simple method is acknowledged. Such a method is the study aim.

Methods: A virtual patient (the virtual respiratory system elaborated previously) exhales air passively through a resistive tube being closed at the maneuver beginning. Then the tube is opened for a moment and closed again. The open airways pressure when the tube is closed (Psat1), i.e. before the tube opening (Psat1) or after the second closing (Psat2) is treated as the alveolar pressure. Airflow (Q) is calculated from the tube resistance and the open airways pressure after tube opening (Psat1) or just before the second closing (Psat2). RAW calculated (RAWc) is equal to (Psat1-Ps). The true RAW is a changeable variable (it depends on such factors as intrapulmonary pressure, airflow rate and direction, etc.), i.e. it is not a parameter of the respiratory system, also our virtual one. Therefore RAWc can be compared with an estimation (RAWe) of RAW. The ratio of the alveolar pressure and the airflow rate during very slow inspiration (or expiration) was assumed as RAWe.

Results: Dead space emptying and oscillations caused by inances significantly disturbed results obtained from Psat1 and Psat2. The use of Psat2 and Psat2 gave more correct results and RAWc appeared to be proportional to RAWe increased by influence of the parenchyma viscosity (note that also FEV1 depends on both RAW and the viscosity). The proportionality coefficient depended on the tube resistance (weakly) and details of RAWe estimation.

Conclusions: The proposed maneuver might support the fixed spirometry because it is easy to perform, requires simple equipment, and does not need cooperation from the patient.

Acknowledgements: The work was partly supported by the grant No. NN518332335 from the Polish Ministry of Science and Higher Education.
**Dialysis Catheters**

**O9 (86)**
WHAT IS THE DIFFERENCE IN DIALYSIS ADEQUACY WHEN SWITCHING CONNECTION PORTS OF A DOUBLE LUMEN TUNNELLED CATHETER?  
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**Objectives:** Catheter dysfunction is a frequent problem with a negative impact on solute clearance during hemodialysis. Reversing the catheter connection mode often results in higher blood flows, but might lead to enhanced recirculation. We evaluated total solute removal (TSR) of different classes of uremic retention solutes during a complete hemodialysis session, once with reversed (RL) and once with correctly connected lines (CL).

**Methods:** Genious® dialysis was performed at midweek in 22 chronic hemodialysis patients, once with RL and once with CL. Blood flow (QB) was in both modes set at the maximum speed tolerated by the system. TSR was determined for urea, creatinine, phosphate and β2M. Also, using a urea single-pool kinetic model, we simulated TSR and reduction ratio (RR) for urea for different percentages of access recirculation and QB during CL versus RL.

**Results:** RR and TSR for urea, creatinine, phosphorus, and β2M were not significantly different in clinical practice between CL versus RL, while maintaining blood flow. In the mathematical model, urea RR did not significantly differ with or without access recirculation, but TSR decreased by 4.5 to 23.3% when changing from CL to RL for an access recirculation of 5 to 25%, respectively. For an access recirculation of 5 or 25%, QB in RL should respectively increase by 6.7 or 52.0%, 8.5 or 72.0%, and 10.0 or 115.2% respectively, for a blood flow of 150, 200 or 250ml/min in CL.

**Conclusions:** We demonstrated that connecting patients to double lumen dialysis catheters in RL does not impair TSR in clinical practice, if the catheter is working well in the RL mode. In the theoretical model, TSR during the RL was dependent upon the obtained blood flow and the presumed degree of recirculation. A nomogram indicating the % increase in blood flow needed in CL as a function of the obtained blood flow and the presumed degree of recirculation is constructed.

**O10 (175)**
OPTIMAL UKRINASE LOCK THERAPY FOR HEMODIALYSIS (HD) TUNNELED CUFFED CATHETER (TCC) THROMBOSIS  
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**Objectives:** A major clinical complication of TCC used for HD is catheter thrombosis or malfunction with blood flow <250 ml/min. Urokinase (UK) therapy for TCC thrombosis is given when mechanical problems such as improper tip positioning or catheter kinking are excluded, but no consensus exists on the adequate treatment to obtain thrombolysis.

**Methods:** To assess an optimal UK lock therapy, 64 HD patients with TCC were selected, median age 74 years (range 65-87), median dialysis vintage 36 months (range 12-61). All patients received warfarin therapy as prophylaxis therapy for TCC thrombosis with target INR 1.8-2.5. In the case of TCC thrombosis or malfunction and INR<1.8, patients were randomized to receive UK lock therapy as follows: 25000 IU for both arterial and venous TCC lines (Group A) vs 10000 IU for both arterial and venous TCC lines (Group B) for 1 hour dwelling. INR, aPTT, fibrinogen, Hb and platelets were checked before each UK administration. All patients had fibrinogen values >200 mg/dL, in case of repeated UK administration fibrinogen levels were checked before and after UK lock.

**Results:** The UK therapy was well tolerated by all patients. During a 5-year follow-up 104 thrombotic events were recorded, median 0.3 event/patient/year. 32 patients were randomized to Group A for 48 thrombotic events. In 6/48 cases (12.5%) UK was able to restore an adequate blood flow for HD, in 42/48 cases (87.5%) an addition of 150000/250000 IU to both arterial and venous TCC lines) was required immediately after the initial lock. In the following 2 HD sessions, 100000 IU UK were needed for both TCC lumen to restore TCC patency. In 24/48 cases (50%) 20000 IU UK were administered for more than 3 of the following dialysis sessions. 32 patients were randomized to Group B for 56 thrombotic events. In all cases the patients obtained the recovery of TCC function and the HD session was carried out, in 19/56 cases (33.9%) 100000 IU UK were needed for both TCC lumen in the following 2 HD sessions more to restore TCC patency. In 4/56 cases (7.1%) 200000 IU were administered for more than 3 of the following dialysis sessions.

**Conclusions:** Our data suggest that 100000 IU of UK in both TCC lumens allow: 1) a better TCC patency than 250000 IU UK in both TCC lumens; 2) the reduction of UK administration rate in the following HD sessions; 3) no bleeding complications.

**O11 (322)**
NOSOCOMIAL INFECTIONS AS A RISK IN HEMODIALYSIS PATIENTS WITH CENTRAL VENOUS CATHETERS  
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Central venous catheters (CVC) are routinely used in the management of hemodialysis (HD) patients (pts). One of the most frequently encountered complications is catheter-related bloodstream infection (CRBI). Stenotrophomonas maltophilia has recently emerged as an important nosocomial pathogen. We describe a HD pts who developed S. maltophilia bacteremia associated with use of CVC.

During a two-year period five pts (3 male, 2 female) on chronic HD program were admitted to our hospital with clinical signs of CRBI. Three pts had tunneled subclavian catheter, one tunneled jugular catheter, and one femoral catheter. Duration time of chronic HD program was between 1 month and 5 years, and duration time of CVC was 1-18 months. All pts had clinical symptoms of high fever and chills during or after the HD, and because we had a suspicion of CRBI we took blood cultures from catheter and peripheral vein and antibiotics were used. Incubation of blood cultures for 48 hours yielded bacterial growth of S. maltophilia. A complete blood count in all pts revealed a white blood cell count of 7,550-24,000 cells/mm³ (70-90% polymorphonuclear cells) and high CRP. Pts had been receiving broad-spectrum antibiotic therapy since the beginning but without effect, and they were changed later according to antibiogram from blood cultures. Antibiotic therapy in all our cases dose not generally cure CRBI so removal of the CVC was recommended, and all CVC were removed. After the insertion of new CVCs all clinical signs of infection disappeared and blood cultures were sterile.

In conclusion, the treatment of CRBI caused by S. maltophilia must include early and accurate diagnosis, use of effective preventive strategies, and appropriate therapeutic clinical decisions about catheter removal.

**O12 (121)**
FLOW, PRESSURE OR RECIRCULATION: WHICH TEST IS BEST FOR DUAL LUMEN CATHETERS?  
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**Objectives:** Although recirculation (R) in catheters is negligible, the origin of several published recirculation studies with R values between 3-5% remains unclear. In the same context it is not clear if a change in arterial (AP) and venous pressure (VP) is always the result of clotted catheter holes or thrombosis at the catheter tip.

**Methods:** All experiments were carried out with a device for renal replacement therapy with blood temperature monitor (4008, FMC, Germany). Hereby the venous catheter tip was connected to a separated hose to prevent recirculation. 5 recirculation measurements with 3 different catheter types were performed at 6 different blood flows QB (n=90). In addition, arterial and venous extracorporeal pressures of each catheter were measured as function of QB in a range between 0-400 ml/min.

**Results:** R and standard deviation (SD) for 6 different QB were R2=+5.40%±0.95% (QB=150ml/min), R3=+3.96%±0.87% (QB=200ml/min), R4=+3.36%±0.30% (QB=250ml/min), R5=+3.45%±0.38% (QB=300ml/min), R6=+3.48%±0.22% (QB=350ml/min), and R7=+3.69%±0.44% (QB=400ml/min). Mean R and SD were R=+3.89%±0.77%. From AP and VP the sum of arterial and venous pressure (PS), a surrogate for flow symmetry of the catheters, was calculated. For blood flows above 300 ml/min the pressure sum becomes more and more negative. PS decreases to values up to PS=+(-70±16) mmHg at effective blood flows of QB=400 ml/min.

**Conclusions:** Even in absence of recirculation, R is 3.4-5.4% for blood flows above 300 ml/min. It follows that thermodilution measurements with dual lumen catheters are hampered by a temperature transfer between the arterial and venous catheter lumen according to the dilution law of Fick. Due to the softness of the catheter material a pressure-induced narrowing of the arterial catheter lumen with increasing negative pressure is found, leading to a negative PS. It follows that an unexpected decrease in arterial pressure with increasing blood flow is only significant for catheter malfunction if any instability of the catheter lumen can be excluded.
Atherosclerosis is an inflammatory disease that starts with intima alterations. Moreover, it is now well accepted that the early stage of the process is the result of interactions between plasma low density lipoproteins that filtrate through the endothelium into the intima, cellular components (monocytes/macrophages, endothelial cells and smooth muscle cells) and the extracellular matrix of the arterial wall. In this study, we presented a model describing the plaque formation and development in the arteries.

**Methods:** The blood flow was simulated by the three-dimensional Navier-Stokes equations, together with the continuity equation. LDL transport in lumen of the vessel is coupled with convective-diffusion equation and inflammatory process equations, together with the continuity equation. LDL transport in lumen of the vessel is coupled with convective-diffusion equation and inflammatory process equations, together with the continuity equation. LDL transport in lumen of the vessel is coupled with convective-diffusion equation and inflammatory process equations, together with the continuity equation. LDL transport in lumen of the vessel is coupled with convective-diffusion equation and inflammatory process equations, together with the continuity equation. LDL transport in lumen of the vessel is coupled with convective-diffusion equation and inflammatory process equations, together with the continuity equation. 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LDL transport in lumen of the vessel is coupled with convective-diffusion equation and inflammatory process equations, together with the continuity equation. LDL transport in lumen of the vessel is coupled with convective-diffusion equation and inflammatory process equations, together with the continuity equation. LDL transport in lumen of the vehicle. The influence of the inlet valve size of the ventricular assist devices on basic hemodynamics parameters. The pulsed Doppler ultrasound and fluid-structure interaction study.

**Objectives:** The main complications associated with the long-term use of Ventricular Assist Devices (VAD) are hemolysis and thrombosis. They are
strongly dependent on high shear stresses, turbulences, flow separations and stagnant flow regions and could be minimized by proper design of VAD. **Materials and Methods:** The determination of this hemodynamic parameters should be carried out indirectly and non-invasively. For this reason blood flow analysis and the hemodynamic changes induced by different size of inlet valve of VAD was carried out using both Pulsed Ultrasound Doppler Velocimeter (PUDV) and Fluid-Structure Interaction (FSI). These methods allow the analysis of changes of hemodynamics parameters, caused by VAD membrane movements too, in whole cardiac cycle and allow to take into consideration the results during design of new shape of VAD. **Results:** A various characteristic of the velocity pattern was observed for each phase of the cardiac cycle. The size of valves and geometry of bowl has a significant influence on the velocity profiles and stresses in connectors as well as inside ventricle. The maximum values of stresses, due to the asymmetric shape of velocity profiles was observed near valves. The separation and stagnant flow region inside chamber also depends on the phase of the cardiac cycle. The blood flow near place of binding of membrane due to high stream flow on inlet (smaller diameter of inlet valve) increases the turbulence and wall stress in chamber between two connectors. **Conclusions:** The PUDV and FSI are very effective tools to obtain a complete velocity profile for each phase of the cardiac cycle. A compromise between the high stresses (shear and Reynolds) and region of flow separations and stagnant flow (low shear stress) during design process in order to improve hemodynamics of VAD must be made.

**018 (82)**

**THE INFLUENCE OF HIGH SHEAR ON THROMBOSIS AND HEMOLYSIS IN ARTIFICIAL ORGANS**

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**Materials and Methods:** A mathematical model describes the concentrations of activated and resting platelets in a continuum approach. Activation of platelets can occur due to chemical stimulation, contact with foreign materials or high shear stresses. The adhesion behavior of platelets to biomaterials is modeled as shear-dependent. Thrombosis experiments with citrated and with heparinized blood were performed for different systems and artificial organs. For the numerical hemolysis computations a strain-based approach was used and the time-dependent deformation of red blood cells was tracked along pathlines. **Objectives:** Blood flow in artificial organs is characterized by unphysiological velocities and often by high shear rates. The activation and adhesion of thrombocytes as well as hemoglobin release from erythrocytes are known to depend on the local shear. It is the aim of this study to experimentally and numerically characterize and determine the influence of high shear rates on blood damage. Results will be shown for a high shear Taylor-Couette system, a tri-leaflet heart valve and a ventricular assist device. For hemolysis the focus is on red blood cell migration effects.

**Conclusions:** The influence of high shear on thrombosis and hemolysis in artificial organs is a complex phenomenon that needs to be studied experimentally and numerically. Further, experimental and theoretical investigations are necessary to better understand the mechanisms of thrombosis and hemolysis in artificial organs.

**THERMAL AND CHEMICAL STRESS**

**020 (248)**

**STEM BASED CELL THERAPEUTIC IMPLANTS: PRODUCTION AND STORAGE**

D. Freimark	extsuperscript{1}, C. Sehl	extsuperscript{1}, T.A. Grein	extsuperscript{1}, C. Weber	extsuperscript{1}, K. Hudel	extsuperscript{1}, B. Glasmacher	extsuperscript{2}, R. Czernacki	extsuperscript{1}

**Materials and Methods:** Human mesenchymal stem cells as cell solution and as encapsulated cells for cyropreservation without losing vitality. In clinical practice, cells can be used as cell solution or encapsulated. Especially, the cryopreservation of encapsulated cells is not well investigated yet. During cryopreservation, cells are exposed to osmotic forces and dehydration. The addition of cryoprotective agents should prevent the cell from damage and maintain cell vitality. Dimethylsulfoxide is often used as a cryoprotective agent, but it has toxic effects. Therefore, dimethylsulfoxide should be avoided for cryopreservation in clinical use. The aim of this project is to develop a method for cryopreservation of human mesenchymal stem cells as cell solution and as encapsulated cells with non-cytotoxic biocompatible cryoprotective agents. Further, a device for the storage of encapsulated stem cells in syringes for clinical use has been developed.

**Objectives:** In cell therapy, stem cells gain more and more importance. The number of patients treated with stem cells increases, so that a solution for cell storage has to be found. For this reason, cells can be conserved via cryopreservation without losing vitality. In practical routine, stem cells can be used as cell solution or encapsulated. Especially, the cryopreservation of encapsulated cells is not well investigated. Yet, during cryopreservation, cells are exposed to osmotic forces and dehydration. The addition of cryoprotective agents should prevent the cell from damage and maintain cell vitality. Dimethylsulfoxide is often used as a cryoprotective agent, but it has toxic effects. Therefore, dimethylsulfoxide should be avoided for cryopreservation in clinical use. The aim of this project is to develop a method for cryopreservation of human mesenchymal stem cells as cell solution and as encapsulated cells with non-cytotoxic biocompatible cryoprotective agents. Further, a device for the storage of encapsulated stem cells in syringes for clinical use has been developed.

**Conclusions:** Alternatives to DMIB have been found and optimal cryopreservation protocols for stem cells as cell solution and as encapsulated cells have been developed. Further on, first experiments with the developed freezing device will be presented.
efficiency or high cost. To address these limitations we have produced a novel supermacroporous gelatin cryogel scaffold with a highly sophisticated ordered anisotropic pore structure. The material was characterized and assessed in vitro for its skin substitution potential.

**Methods:** The cryogel was characterized morphologically by confocal laser scanning and scanning electron microscopy. The dynamic elasticity and viscosity were assessed to characterize its mechanical properties. The cryogel was seeded with primary human fibroblasts or keratinocytes to assess its cytotoxicity and biocompatibility. The rates of cellular migration, proliferation, and protein deposition were assessed immunohistochemically over a 28-day period. Keratinocytes were also seeded onto the cryogel scaffold to assess the ability to form a bilayered artificial skin. The performances of a gelatin cryogel were compared with those of a commercially available dermal regeneration template, Integra®.

**Results:** The gelatin cryogel scaffold demonstrated a supermacroporous, highly sophisticated, ordered, anisotropic pore structure. The material was morphologically comparable to Integra®. The cryogel was found to be biocompatible and non-toxic to human skin cells, supporting active cellular migration and proliferation. The keratinocytes formed a continuous differentiated layer on the surface of the gelatine cryogel scaffold, mimicking the native skin bilayered structure.

**Conclusions:** The large size of interconnected gradient pores, biocompatibility and small production costs of gelatine cryogels make them a promising material for tissue engineering and regenerative medicine, to treat burns and chronic wounds.

**Acknowledgements:** The work was funded by MARIE CURIE TOK FP6 MKTI-CT-2006-42768 grant.

### O22 (283)

**HOW DOES HYPOTHERMIA PROTECT CARDIOMYOCYTES DURING CARDIOPLEGIC ISCHEMIA?**

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**Objectives:** Insufficient myocardial protection is still considered a significant cause of in-hospital mortality after cardiac surgery. The purpose of our study was to investigate underlying basic mechanisms of cardioplegic cardioprotection during hypothermic and normothermic ischemia in an H9c2 cardiomyocyte cell culture model.

**Methods:** We cooled cardiomyocytes to 20°C for 20 min; during this time cardiac arrest was simulated by oxidative damage with 2 mM H2O2 and cardioplegic solution, followed by rewarming to 37°C. Later on we analyzed cardiomyocyte viability (trypan blue staining), inflammation (Cox-2 and pERK 1/2 expression in Western blot analysis) and expression of Akt survival protein (Western blot technique).

**Results:** Hypothermia increases cell survival of cardiomyocytes after cardioplegic ischemia as demonstrated in significantly higher cell viability and less cell death in these cells compared to normothermic H2O2 damaged cardiomyocytes. As a possible underlying cellular mechanism we found that during cold cardioplastic ischemia ERK 1/2 enzyme is less phosphorylated than under normothermic cardioplastic ischemia. This is in line with significantly diminished Cox-2 expression during cold cardioplastic ischemia. Moreover, hypothermic cardioplegia preserved cell survival by up-regulation of Akt transcription factors in cardiomyocytes.

**Conclusions:** In the present cell culture study we clearly demonstrated that hypothermia exerts additional protection for cardiomyocytes during cardioplastic ischemia. The understanding of underlying basic mechanisms is evident to improve current techniques of myocardial protection.

### O23 (285)

**DEEP HYPOTHERMIA LEADS TO CELLULAR STRESS AND ENDOThelial DYSFUNCTION IN A COCULTURE MODEL OF ENDOThelial CELLS AND MACROPHAGES**

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**Objectives:** During cardiac surgery with cardiopulmonary bypass (CPB), patients are often cooled to a temperature of 14-18°C. Cardiopulmonary bypass triggers a systemic inflammatory response. We hypothesized that dynamic temperature changes may influence this inflammation by activating leucocytes. The aim of our studies was therefore to investigate the effects of deep hypothermia and rewarming on morphological changes, inflammatory response and cellular stress in a coculture model of endothelial cells and macrophages.

**Methods:** Primary human umbilical vein endothelial cells (HUVECs) and subconfluent THP-1 cell line were exposed to dynamic temperature changes analogous to the clinical settings similar to pediatric cardiac surgery: deep hypothermia (20°C), slow rewarming (30 min up to 37°C) and normothermia (48h at 37°C). To imitate inflammatory response after CPB, the coculture was stimulated with 500 U/mL TNF-α. Cell viability, cell morphology, expression of IL-6, IL-8 and MCP-1, as well as intracellular Reactive Oxygen Species (ROS) and Adenosin-Triphosphat (ATP) content were investigated.

**Results:** Deep hypothermia had no influence on cell viability and the inflammatory response after TNF-α stimulation. Interestingly, hypothermia increased endothelial cell layer permeability and induced significant higher intracellular ROS concentrations and less ATP content compared to cells kept under normothermia.

**Conclusions:** In a new coculture cell model with endothelial cells and macrophages, hypothermia and rewarming led to a dysfunction of the endothelium cell barrier, an increase in mitochondrial oxidative stress combined with an ATP decrease. Therefore, hypothermia treatment should be performed with caution as underlying hypothermia induced mechanisms are not fully understood.

### O24 (126)

**SUSTAINED DELIVERY OF PROTEINS FROM ELECTROSPUN FIBRES**

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**Objectives:** Electrospun nanofibres are excellent as scaffolds for tissue engineering, especially if functionalized with proteins to direct cell behavior. However, commonly used organic solvents for electrosprining denature the proteins. Here, we describe instead a process to produce such fibre mats in aqueous conditions, capable of sustained delivery of proteins.

**Materials and Methods:** The model proteins albumin, fibrinogen, alkaline phosphatase (AP) and basic fibroblast growth factor (Bfgf) were blended with the polymer solution containing polyethylene oxide (PEO) and the crosslinkers prior to electrosprining. The fibres were cross-linked and observed with SEM. Changes in the protein secondary structure were observed with FTIR-spectroscopy. For the release kinetic studies, the protein concentration was measured using standard Bradford assay. AP activity was measured using p-nitrophenyl phosphate as the substrate. In vitro studies were carried out using NIH-3T3 cells.

**Results:** The protein functionalized fibres had a diameter of around 250 - 300 nm. Qualitative analysis of the amide I band of the FTIR spectra was used to fine-tune the process to limit protein denaturation. The proteins showed a biphasic release profile, with an initial burst release for the first 2 days, with a subsequent steady release phase. AP retained around 50% activity on release, even after a week. Initial in vitro experiments showed that the fibre mats were not cytotoxic. It was also possible to get the ST3-cells to attach and grow on the fibre mats functionalized with the adhesion promoting protein fibrogen.

**Conclusions:** A method to functionalize fibres with proteins by electrosprining in aqueous conditions was developed. Activity of the released proteins was retained even after a week. The fibre mats were also found to be compatible with cells in the in vitro studies. This project was partially funded by DFG for the cluster of excellence REBIRTH (EXC 62/1).

### O25 (166)

**PDMS ENCAPSULATING RELEASING DEXAMETHASONE AND ITS INFLUENCE ON THE GROWTH OF FIBROBYTES**

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**Objectives:** Cochlear Implants (CI) are an established method to treat deafness. The CI implies the insertion of a silicon-embedded electrode into the middle ear. The implantation often causes acute inflammatory reactions and fibrosis. Dexamethasone is a potent synthetic glucocorticoid drug commonly administered systemically to prevent inflammatory reactions. A local administration of Dexamethasone would be preferable as an adjuvant therapy when inserting a CI and for the long-term prevention of fibrosis.© 2010 Wichtig Editor - ISSN 0391-3988

**Conclusions:** A method to functionalize fibres with proteins by electrosprining in aqueous conditions was developed. Activity of the released proteins was retained even after a week. The fibre mats were also found to be compatible with cells in the in vitro studies. This project was partially funded by DFG for the cluster of excellence REBIRTH (EXC 62/1).
Methods: A two component pourable platinum-catalyzed cross-linking polydimethylsiloxane (PDMS) system is used. Dexamethasone is dispersed in the PDMS system prior to cross-linking. Cylindrical matrices with diameters of 300 and 500 µm were produced by an injection moulding-like process. Dexamethasone solutions (1ng/µl ~ 500ng/µl) and pieces of dexamethasone loaded silicone matrices in cell medium were incubated in with Human fibroblasts for 5 days and the cell count was determined using a Neubauer haemocytometer. The dexamethasone concentrations were determined using HPLC.

Results: The growth of fibroblasts was inhibited with dexamethasone concentrations lower than 100 µg/ml. With the dexamethasone loaded matrices a controlled release over 50 days was observed. The amount of released dexamethasone minimizes the growth of fibroblasts in vitro significantly. Furthermore no fibroblasts attached to the dexamethasone loaded silicon matrices could be detected.

Conclusions: Dexamethasone can be successfully embedded to and released from a PDMS matrix. Furthermore, the released doses of dexamethasone effectively minimize the growth of fibroblasts.

ARTIFICIAL KIDNEY

O26 (347) AMBULATORY ULTRAFILTRATION: DRY WEIGHT ALL THE TIME
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Steady euvoeulmia in ESRD patients is almost impossible to maintain with currently available treatment options. Inability to maintain constant dry weight causes discomfort, uncontrollable hypertension and intra- and post-dialytic hypotension that compromise solute removal. We are studying an ambulatory ultrafilter that will maintain invariant dry weight and when used in conjunction with twice-weekly in-clinic dialysis can provide adequate solute removal. Blood is flowed through microfluidic channels bounded by filters that consist of twice-weekly in-clinic dialysis can provide adequate solute removal.

O27 (322) HEALTH-RELATED QUALITY OF LIFE IN END-STAGE KIDNEY DISEASE PATIENTS
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Objectives: Measures of HRQOL have a significant predictive value for patient survival and hospitalizations, especially in patients with chronic kidney disease (CKD).

Methods: A review of the major studies performed in Serbia, other countries from Europe and USA is presented.

Results: Patients with CKD had higher SF-36 scores than those reported for the adult population. Hemoglobin level predicted both physical and mental domains of the SF-36. Longitudinal studies are needed to define at-risk periods for decreases in HRQOL during progression of CKD. HD and PD are associated with similar HRQOL outcomes at 1 yr. Generic HRQOL in two domains improved more for HD patients. However, for ESRD-specific HRQOL, results were not consistent; some domains were better for PD patients whereas others were better for HD patients. Kidney transplantation offers better HRQOL. Depression occurs in about 20-30% of dialysis patients. This is important not only because of the negative impact of depression on quality of life but also because it is now well established that depression can significantly affect the morbidity and mortality of patients with ESRD. Satisfaction with sexual relationships showed marked deterioration in all age groups. Those aged greater than 65 scored significantly better than younger patients on dialysis stress scales, and were generally more satisfied with life.

Conclusions: There is no significant difference in HRQOL for prevalent ESRD patients treated with hemodialysis or peritoneal dialysis. It will be important to determine if this finding holds true for incident patients treated with hemodialysis or peritoneal dialysis. The high prevalence of anemia, hypoalbuminemia, and depressive symptoms at dialysis therapy initiation suggests the need for more aggressive and broader spectrum pre-ESRD care. HRQOL of Serbian dialysis patients was similar to that in other Balkan countries, however, lower than in Italy, Spain and France, probably due to the higher degree of malnutrition and living and health care standards in these countries.

O28 (233) LANTHANUM CARBONATE RETARDS THE PROGRESSION OF VASCULAR CALCIFICATION AND Atherosclerosis in Uremic apolipoprotein E DEFICIENT MICE
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Rapid progression of atherosclerosis and vascular calcification in chronic kidney disease (CKD) is probably favored by the associated disorder of mineral and bone metabolism (MBD). The purpose of the study was to compare the effect of phosphate binder lanthanum (La) carbonate with that of sevelamer-HCl on arterial calcification and atherosclerosis and also on bone structure and turnover in mice with chronic renal failure (CRF).

Conclusions: CRF or sham operated apolipoprotein E-deficient (apoE-/-) mice were randomized to one non CRF and three CRF groups and fed with standard diet alone (one non CRF and one CRF group), or with same diet supplemented with either 3% lanthanum carbonate (La3%; CRF) group or 3% sevelamer-HCl (Sev3%; CRF) group.

Compared with unsupplemented control CRF, both the La3% and Sev3% supplemented CRF mouse groups displayed a decrease of serum phosphorus, a reduction of arterial calcification at both intimal (plaque) and medial (non-plaque) sites of the aortic root, and a lower degree of atherosclerosis at the thoracic aorta site. These improvements were associated with a reduction of aortic plaque collagen I expression in response to either phosphate binder, and in nitrotyrosine expression in response to Sev3% only. Proteomic analysis showed that several peptide peaks were significantly modified by CRF state, at least one peak by Sev3%, and no peak by La3% supplementation. Finally, increased mineral apossition and bone formation rates in unsupplemented CRF mice were reduced by Sev3%, but not La3% treatment.

Both La and Sev retarded the progression of vascular calcification and atherosclerosis in CRF apoE-/- mice. These effects could be mainly due to the control of hyperphosphatemia by the two phosphate binders, and likewise the reduction of arterial collagen I expression. The effect of La differed however from that of Sev in that it did not appear to exert its vascular effects via changes in oxidative stress or bone remodeling in the present model.

O29 (220) ENDOTOXINEMIA ACTIVITY IN POST-SURGICAL PATIENTS, ROLE OF ENDOTOXIN ACTIVITY ASSAY AND POLYMYXIN-B IN EARLY MANAGEMENT
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Objectives: The aim of this study was to evaluate the ability of the Endotoxin Activity (EA) assay to determine the need for early intervention of endotoxemia using polymyxin-B based hemoperfusion (PMX-DHP) on septic patients. The secondary end-point is to highlight the major incidence of endotoxin activity in transplant patients.

Methods: From April 2008 to October 2009, forty-one patients after surgical period with diagnosis of SIRS were enrolled in this study. Cause of surgery: major abdominal surgery 22 patients(pt); liver transplant 14pt; kidney transplant 3pt and lung transplant 2pt. Nineteen patients had a high EA level (≥ 0.6) and were treated with PMX-DHP every 24 hours until the EA level was low (< 0.4). The remaining twenty-two had EA levels <0.60 and received standard therapy only.

Results: Twenty-two (55%) showed a low endotoxin activity level (EA < 0.6) at the first examination. These levels did not significantly change after 24 hours.
Abstracts: XXXVII Annual ESAO Congress, 8-11 September 2010, Skopje - R. Macedonia

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except one patient. This patient showed an EA of 0.62 after 24 hours and was then shifted to the treatment group. Microbiological findings of these patients in the low endotoxin level group showed the presence of Gram-positive infections in 14 of 22 patients, 5 infections of mycobacterium and three fungal infections. Of the 19 patients with no infections (15% with EA > 0.60, 25% had abdominal surgery; 70% had liver transplant; 66% kidney transplant and 50% lung transplant. Among patients showing EA<0.6, seven patients (median EA=0.64 [0.62-0.87] required two PMX-DHP treatments, nine patients (median EA=0.845 [0.74–1.08]) required three treatments and three patients (median EA=0.985 [0.72–1.23]) required four treatments in order to reach EA<0.4 condition (median 0.328 [range [0.22-0.48]). No adverse events were observed during the hemoperfusion treatments performed. At the end of the PMX-DHP therapy, a statistically significant improvement in the hemodynamic parameters, Mean Arterial Pressure (MAP) and Heart Rate (HR), were observed. White blood cells count significantly decreased and the PaO2/FiO2 ratio increased. Median SOFA scores also decreased from 7 (range [3–13]) to 4 (range[1–12]) at the end of the therapy.

Conclusion: The EA assay can identify patients eligible for PMX-DHP treatment and aids its therapeutic dosing.

O30 (48)

EFFECTS OF APOLIPOPROTEIN E DEFICIENCY AND CHRONIC RENAL FAILURE ON BONE STRUCTURE AND FUNCTION IN MICE
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Chronic kidney disease (CKD) is associated with a disorder of mineral and bone metabolism (MBD). This is of clinical concern because of the potential contribution of CKD-linked MBD to the high risk of cardiovascular disease (CVD) and fractures, which may be interrelated. The purpose of the study was to investigate the effects of chronic renal failure (CRF) on bone metabolism of apolipoprotein E-deficient (apoE/-) mice with accelerated vascular disease as compared to C57/BL/6 wild type (WT) mice. CRF or sham 10-week-old female apoE/- and WT mice were randomly assigned to 4 groups and fed with standard diet. 8 weeks later, animals were euthanized and serum and femoral bones were sampled for examination by bone histomorphometry (BHM) and 3-dimensional microtomography (µCT). Atherosclerotic and calcified lesions in the aortas of apoE/- mice were analyzed. We first found an increase in bone volume (BV/TV) and trabecular thickness in apoE/- mice as compared to WT mice (by 31.5% and 6%, respectively). CRF led to a further augmentation of BV/TV (by 12% in WT and by 77.2% in apoE/- mice). Three-dimensional µCT structural analysis revealed an increase in Connectivity Density (Conn.D) and Structure Model Index (SMI) in apoE/- mice. We observed an increase in osteoid surface and osteoblastic parameters in CRF mice while resorption parameters were less augmented by CRF in apoE/- mice. Finally, we found positive correlations between atheroerotic lesions and bone volume parameters, and between plaque calcification and osteoclast parameters in apoE/- mice.

In conclusion, apolipoprotein E deficiency in mice is associated with an increase in bone mass and volumetric mineral density. Bone mass is further increased, whereas bone mineral density is decreased, in response to CRF. Our finding of correlations between changes in bone structure suggests a link between bone and vascular disease.

O31 (184)

ANNUAL INCREASE OF SKIN AUTOFLUORESCENCE PREDICTS SURVIVAL OF HD PATIENTS
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Objectives: The aim of this study was to measure the Advanced Glycation End Products (AGEs) accumulation in a period of 18 months and to predict the seasonal fluctuation of Skin AF, 27.8 (2.49-309) for the Skin AF level at the start of the study of independent predictors of survival of HD were 19.9 (2.68-148) for the annual change of Skin AF, 27.8 (2.49-309) for the Skin AF level at the start of the study and 1.03 (1.01-1.06) for CRP.

Conclusions: Apart from Skin AF and CRP the annual rate of increase of skin AF as a measure of AGE accumulation can be used to predict survival in HD patients.

CARDIOVASCULAR DEVICE ENGINEERING

O32 (329)

SHEAR RATE AND PLATELET DEPOSITION IN WHOLE BLOOD
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Objectives: Objective of the research is to quantify the shear rate, which is likely to favor the deposition of platelets in whole blood. This is important, because cardiovascular implants, such as assist devices, frequently cause thromboembolic complications. These still severely limit their clinical application. One reason for these complications is the activation of platelets by high mechanical shear stress followed by a flow of low shear stress in the vicinity of a foreign material.

Methods: Whole blood is sheared in a gap between two parallel glass plates with a diameter of 35 mm and a distance of 50 μm. The lower plate remains stationary, while the upper plate is rotated. This shearing device is attached to an inverted microscope (Fluovert FU, Leica) and the deposition of the platelets is recorded and analyzed. The platelets are stained with Mecaprine and are illuminated by ultraviolet light with 355-425 nm wavelength and respond with an emission of green light with 496 nm wavelength.

Results: When the deposited platelets per time unit are plotted as a function of shear rate, a peak is observed at a shear rate around 100 1/s.

Conclusions: This is interpreted as the interaction of two modes of transport of thrombo-active substances in blood: diffusive and convective. When both modes combine in a specific relation, an optimal transport is achieved, which favors the generation of a thrombus.

O33 (259)

TRANSCUTANEOUS ENERGY TRANSFER (TET) SYSTEM WITH NOVEL EXTERNAL CARRIER SYSTEM
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Objectives: Percutaneous drivelines cause infections and technical problems. To minimize complications and increase patient's mobility, a transcutaneous energy- and data transfer system is to be developed with high tolerance of transmission and a convenient external carrier system.

Methods: The inductive TET includes two coreless coils (D=65mm) with external and internal control units equipped with accumulators. An integrated controller provides telemetry data processing and control of the implant. Wireless data transfer is enabled by using RF transmission and a proprietary protocol. The performance is verified in a body simulator in vitro and in acute animal studies. A positioning assistance is developed for exact placement of the external coil. A performance is verified in a body simulator in which the external transmitter coil is integrated. The carrier system is verified in a body simulator

Results: The developed TET is able to transmit up to approximately 22 Watt through the tissue. Bi-directional data communication is improved to a rate of 500 kbits/sec, where the external receiver is allowed to be up to 3m distant.
to the patient. The maximum efficiency of the system is approximately 78% at 5 mm distance between the coils and 61% at 15 mm. Displacement of the coils reduces the efficiency and leads to a warming of the external transmitter electronic. No warming is measured between the coils and the implanted components under any operating condition. The positioning system enables easy alignment of the external coil with an accuracy of 1 mm.

**Conclusions:** The TET shows reliable transmission at horizontal and vertical displacements up to 20mm. Transmitted energy is automatically adapted to the demand of the implanted device. Twisting of the flexible coils did not influence the transmission appreciably.

**O34 (258)**

**INFLUENCE OF THE AORTIC ROOT COMPLIANCE ON HEMOLYSIS AND CLOSING DYNAMICS OF HEART VALVE PROSTHESSES: AN IN VITRO STUDY**


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**Objectives:** Mechanical heart valve prostheses are known to activate the coagulation system and may cause hemolysis. Both are particularly depending on the closing behavior and may even be boosted by cavitation. In this study we investigated the correlation between hemolysis and the compliance of the aortic root in a novel, pulsatile in vitro blood tester.

**Methods:** In total 24 hemolysis tests were carried out for 2 mechanical heart valves, the St. Jude Medical bileaflet prosthesis and the novel trileaflet prosthesis TRIFLO. The tests were performed in a newly developed pulsatile blood tester with 2 different setups: a stiff and a compliant aortic root. 6 tests were performed per valve and setup. They were run for 3 hours each with 600 mL fully heparinized porcine blood at identical, physiological pressure and flow conditions of 120/80 mmHg and 5 l/min. The heart rate was set to 70 bpm with a systolic/diastolic ratio of 35%. Samples were taken hourly and free plasma hemoglobin was measured. To evaluate the changes in hemolysis, valve closing sounds were recorded via a hydrophone (type 8103; Bruel&Kjaer) and analyzed online. High-speed videos with a blood analog transparent fluid were analyzed for measuring the closing speed.

**Results:** For the TRIFLO free plasma hemoglobin increased 13.4 for the flexible and 19.3 mg/dL for the stiff setup during the 3-hour test. FFT spectra and closing speed showed slight differences between the two setups. For the SJM valve free plasma hemoglobin was 22.2 mg/dL in the flexible and 42.7 mg/dL in the stiff setup. The closing speed was almost twice as high with the rigid aorta. The mean closing pressure doubled and the spectrum showed elevations around 15 and 18.5 kHz.

**Conclusions:** For the TRIFLO the hemolysis was almost independent of the aortic stiffness. The tests showed that hemolysis in the SJM heart valve may be elevated in a stiff, e.g. calcified aortic root. The reason for this might be cavitation induced by higher closing speed.

**O35 (277)**

**STUDY OF A VALVELESS COUNTERPULSATION HEART ASSIST DEVICE WITH TWO MEASUREMENT METHODS**

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**Objectives:** The flow field investigation of blood pumps allows a first estimation of the risk regions of thrombus formation. The pump under consideration is a novel long-term implantable counterpulsation device (CPD). The CPD is a single port, valveless 32ml, stroke volume blood chamber attached to the subclavian artery. It can be implanted subcutaneously, similar to a pacemaker.

**Materials and Methods:** The time resolved flow investigation of the CPD was done by Particle Image Velocimetry (PIV) to obtain the flow field in the center of the CPD (PVC) to obtain the near wall flow. Both methods were realized on a setup, consisting of a Windkessel and the CPD. Especially a 1in2 operation mode (one pump action every two heart beats), which is more prone realized on a setup, consisting of a Windkessel and the CPD. Especially a 1in2 central plane and Wall-PIV to obtain the near wall flow. Both methods were done by Particle Image Velocimetry (PIV) to obtain the flow field in the artery. It can be implanted subcutaneously, similar to a pacemaker.

**Results:** During ejection phase the fluid flows towards the port of the CPD uniformly, resulting in a good washout. During filling phase a steadily rotating vortex expanding over the complete blood pump, with a permanently moving center, is observed. This large vortex was sustained for over 1.2 seconds of the hold time after the completion of CPD filling providing adequate washing even in a 1in2 operation mode.

**Conclusions:** The investigated CPD has a good washout of the whole pump volume. Regions of stagnation are inhibited by a persistent steady rotating vortex. A low risk of thrombus formation in the blood chamber is expected.
patients got an aortic coronary bypass in combination (2.3 grafts per pts); 1 patient developed middle aortic regretion. Mortality rate was 9.5% (4pts). Follow-up period 1-19 months.

Conclusions: Real sternal aortic valve bio-prosthesis ensures hemodynamic improvement with a small transvalvular gradient in patients. It can be implanted even in patients with small root or with bicuspid valve, with good clinical outcome.

**Vascular Tissue Engineering**

**O38 (41)**

SUCCESSFUL BEAGLE PULMONARY VALVE REPLACEMENT OF COMPLETELY AUTOLOGOUS VALVED-CONDUITS WITH THE SINUS OF VALSALVA (BIOVALVES)

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Objectives: We developed autologous prosthetic implants by simple and safe in-body tissue architecture technology. We present the first report on the development of autologous valved conduit with the sinus of Valsalva (BIOVALVE) by using this unique technology, and its subsequent implantation in the pulmonary valves in a beagle model.

Methods and Results: A mold of BIOVALVE organization was assembled using 2 type silicone rods with a small aperture in a tetrasilaf shape between them. The concave rods had 3 projections that resembled the protrusions of the sinus of Valsalva. The molds were placed in the dorsal subcutaneous spaces of beagle dogs. After 4 weeks of implantation, autologous connective tissues completely covered the surface of the molds. BIOVALVES with 3 leaflets in the inner side of the conduit were obtained after removing the molds. These valves had adequate burst strength, similar to that of native valves. Tight valvular coaptation and sufficient open orifice area were observed in vitro. These BIOVALVES were implanted to the main pulmonary arteries as allogenic conduit valves. Postoperative echocardiography demonstrated smooth movement of the leaflets in Valsalva. The molds were placed in the center of culture dish of 52 mm internal diameter to make a doughnut-shaped space. The culture dish was placed on a tilted plate, which rotates to make a vortex flow around the silicone plate with the swing motion. Variations were made on the diameter (20 mm, 30 mm, and 40 mm) of the silicone disk and the rotational speed (2.1 rad/sec, 5.2 rad/sec) of the swinging plate, which tilted with 0.1 rad from the horizontal plane. Four kinds of cells were cultured in the vortex flow of Dubbeco’s Modified Eagle’s Medium for seven days: C212 (mouse myoblast), L6 (rat skeletal muscle cell), A7r5 (rat aortic smooth muscle cell), and CS-2P2-C75 (primary normal porcine aortal endothelial cell). The volume of the medium is 2 ml for 40 mm diameter, and 3 ml for 30 mm and 20 mm diameter to fill the doughnut-shaped space between the silicone disk and the rim of the dish. The orientation of cells was observed with a phase-contrast microscope. The experiments show the following results. The orientation of cells depends on flow and on kinds of cells, A7r5 and CS-2P2-C75 line along to the streamline of the flow. C212 and L6 adhere along the direction of flow in the first stage, and tilt to the perpendicular direction to the flow differentiating to myotubes with fusion in the second stage.

Conclusions: BIOVALVES could have potential applications in the treatment of patients with valvular disease in the future.

**O39 (37)**

WATER-SOLUBLE ARGATROBAN FOR ANTIHROMBOGENIC SURFACE DESIGN OF TISSUE-ENGINEERED CARDIOVASCULAR TISSUES

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Objectives: Argatroban, which is one of the most powerful anticoagulants but also promote cell proliferation, was evaluated for coating on tissue engineered vascular grafts. In this study, we evaluated the cell orientation to make engineered tissue. For example, anionic argatroban was synthesized by neutralization of argatroban from its alkaline solution, dialysis and freeze-drying. The obtained anionic argatroban could dissolve in water easily. Surface chemical compositional tuning in vivo behavior of electrospun, small diameter conduits of different porosity in rat model.

Methods: Vascular grafts (length 15 mm, inner diameter 1.5 mm) were fabricated by electrospinning using polyether-urethane. Fine mesh grafts (void fraction 70%, n=28) or coarse mesh grafts (void fraction 80%, n=28) were implanted for either 7 days or 1, 3 or 6 months into the abdominal aorta. Retrieved specimens were evaluated by histology.

Results: Within 1 month, grafts with larger pore size revealed significant increased host cell immigration in all areas of the conduit wall. Long-term implants of coarse mesh conduits showed increased intimal hyperplasia and synthesis of collagen.

Conclusions: Electrospun polyurethane conduits with greater porosity will decrease cell ingrowth. However, increased synthesis of extracellular matrix promotes compliance mismatch and resultant neointimal hyperplasia.

**O40 (74)**

EFFECT OF VORTEX FLOW ON ORIENTATION OF CULTURED CELLS

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Cell culture technique has been progressed and several techniques have been studied to arrange cell orientation to make engineered tissue. For example, myoblasts have been clinically applied to ischaemic cardiomyopathy in the field of regenerative medicine. A technique for accelerating orientation of cells has been developed to make muscle tissue in vivo and in vitro. Control methodology for orientation of cells would be applied to regenerative tissue technology. The effect of vortex flow on cell culture has been studied in vitro in the present study. A silicone disk of 3 mm thick was placed in the center of culture dish of 52 mm internal diameter to make a doughnut-shaped space. The culture dish was placed on a tilted plate, which rotates to make a vortex flow around the silicone plate with the swing motion. Variations were made on the diameter (20 mm, 30 mm, and 40 mm) of the silicone disk and the rotational speed (2.1 rad/sec, 5.2 rad/sec) of the swinging plate, which lifts with 0.1 rad from the horizontal plane. Four kinds of cells were cultured in the vortex flow of Dubbeco’s Modified Eagle’s Medium for seven days: C212 (mouse myoblast), L6 (rat skeletal muscle cell), A7r5 (rat aortic smooth muscle cell), and CS-2P2-C75 (primary normal porcine aortal endothelial cell). The volume of the medium is 2 ml for 40 mm diameter, and 3 ml for 30 mm and 20 mm diameter to fill the doughnut-shaped space between the silicone disk and the rim of the dish. The orientation of cells was observed with a phase-contrast microscope. The experiments show the following results. The orientation of cells depends on flow and on kinds of cells, A7r5 and CS-2P2-C75 line along to the streamline of the flow. C212 and L6 adhere along the direction of flow in the first stage, and tilt to the perpendicular direction to the flow differentiating to myotubes with fusion in the second stage.
**Transplantation**

O43 (155)

**ASSESSMENT OF BIODEGRADABLE SYNTHETIC VASCULAR PROSTHESIS IN THE PIG CAROTID ARTERY MODEL**

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- **Objectives:** A functional autologous tubular tissue, termed “biotube”, has been developed as a small-caliber vascular graft. Its histological and mechanical evaluations were performed after 2 year implantation.
- **Methods and Results:** Biotube with “anastomotic reinforcement cuffs” was prepared by embedding a silicone rod (diameter, 3 mm; length, 30 mm) as a mold in a dorsal subcutaneous pouch of a rabbit (weight: ca. 2 kg). The rod was covered at both ends with 2 pieces of polyurethane sponge tubes (length, 3 mm) and it was removed when the graft was harvested. The biotube had a homogenous thin connective tissue wall (thickness: 76 um) that was primarily composed of collagen and fibroblasts. The resulting cuff impregnated biotube after argatroban loading was auto-implanted in the carotid artery for 26 months. Neither anastomotic nor anticoagulant agents were administered except for the intraoperative heparin injection. Follow-up angiography showed that no instance of aneurysm formation, rupturing or stenosis during implantation. After implantation, wall thickness in harvested biotube (212 um at anastomosis portion, 150 um at mid portion) was similar to native one (189 um). Elastic modulus of biotube after implantation increased from 510 kPa to 1750 kPa, which is about two times larger than that of native artery. The luminal surface was completely covered with endothelial cells on the newly developed lamina elastica interna. The regenerated vascular walls comprised multilayered smooth muscle cells and dense collagen fibers with regular circumferential orientation. The biotube with “anastomotic reinforcement cuffs” was developed as a small-caliber vascular graft. Its histological and mechanical properties, no aneurysm formation and similar short-term patency compared to Eptfe grafts. Rapid, good endothelialization and cell ingrowth confirms the hypothesis of vascular tissue engineering. Despite good early results long-term follow-up is required before clinical application.

**O44 (202)**

**DEVELOPMENT OF A CAPD CATHETER WITH PROTECTIVE SLEEVE TO AVOID INFECTION**

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- **Objectives:** In Western countries very few end-stage renal failure patients receive peritoneal dialysis (PD) compared to hemodialysis treatment. This is in part due to the relatively high risk of exit-site infections which can lead to peritonitis. The infection is caused by the growth of a biofilm, which starts at the exit site of the catheter. The use of drug-eluting catheters or catheters with local silver particles has not solved the problem. Objective of this project is to develop an infection-resistant catheter which uses a mechanical method to permanently prevent the downgrowth of the biofilm.

**Methods:** The presented catheter is equipped with a protective sleeve, which surrounds the catheter in the skin penetrating area. In the subcutaneous region the sleeve is folded and the proximal end is sealed to the catheter. It fits snugly on the catheter but can still be moved. The sleeve is made of PUR by dipping on a mandril and its surface is coated with PET fibers to enable ingrowth of connective tissue. After the implantation the protective sleeve is then slowly pulled towards the distal end by means of a small traction device. The pulling speed is kept at a rate of 1 mm per week so it can grow out of the skin but still moves faster than the biofilm grows towards the inside of the body. The traction device is developed using a 3D-CAD system and the prototype is then manufactured by laser sintering technique.

**Results:** In a first key experiment in goats the function of the protection sleeve could be shown. For a new series of experiments the pig is chosen, because of the greater similarity of its skin to the human skin. So far 7 have been implanted. In the ongoing animal experiments the current catheter setup is implanted next to standard Tenckhoff catheters as control group in minipigs.

**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.

O45 (46)

**IMMUNOHISTOCHEMICAL LOCALIZATION OF BMP-7 AND GREMLIN IN SPECIMENS OBTAINED AFTER GRAFTECTOMY**

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- **Objectives:** Bone morphogenetic protein-7 (BMP-7), and its antagonist gremlin, which is a downstream mediator of TGF-beta, participate in kidney development and regulate growth, differentiation, chemotaxis and apoptosis of various cell types. In the present study, we investigated expression of BMP-7 and gremlin in kidney graftectomy specimens.

**Materials and Methods:** Thirty-one graftectomy specimen were included in this investigation. Graftectomy was performed in 18 patients with chronic allograft nephropathy (CAN) who developed acute rejection after return to dialysis (Group 1), and for other reasons in 13 patients (2 renal vein thromboses, 2 renal artery thromboses, 3 renal artery aneurysms, 2 cases of acute rejection in patients with life-threatening infection, 3 FSGS recurrences and 1 bleeding from renal artery) (Group 2). BMP-7 and gremlin immunostaining intensity was evaluated semiquantitatively (0, negative; 1 weak; 2, moderate; 3, strong staining).

**Results:** Out of 31 patients, aged 17-65 (average 44), there were 14 female patients. We observed strong BMP-7 immunostaining in Group 2 with average score 2.92 (range 2-3, median 3), and gremlin 0.69 (0-1, median 1). Group 1 had lower BMP-7 expression with score 1.72 (1-3, median 1). All samples from the Group 1 stained negative for gremlin.

**Conclusions:** BMP-7 immunostaining demonstrated significantly stronger protein expression in samples without chronic changes in allograft tissue. Surprisingly, gremlin expression was demonstrated only in grafts removed for “non-CAN” reasons. It is contrary to its well-known role in fibrosis. However, it is possible that immunosuppressive treatment influences gremlin expression. Further studies are needed to elucidate this dilemma.
EFFECTS OF ASYNCHRONOUS PULSATILE MECHANICAL CIRCULATORY SUPPORT ON REGIONAL ORGAN FLOW. EXPERIMENTAL STUDY WITH COLORED MICROSPHERES

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Objectives: To measure the regional blood flow in different organs in healthy minipigs with a VAD working in asynchronous mode, first in conditions of total unloading and then in conditions of partial support, at baseline and during partial support.

Methods: We studied 10 minipigs with a VAD working in asynchronous mode, first in conditions of total unloading (PVAD) and then in conditions of partial support (MPVAD). In PVAD, systemic blood flow was maintained at approximately 55% of baseline while in MPVAD, flow was maintained at approximately 30% of baseline. We used a novel technique of colored microspheres to measure regional blood flow. The study was approved by the institutional ethics committee.

Results: We observed a significant increase in regional blood flow in the heart, liver, and kidney in both PVAD and MPVAD. In contrast, there was a significant decrease in regional blood flow in the brain and skeletal muscle in both conditions.

Conclusions: The results suggest that asynchronous pulsatile mechanical circulatory support is effective in maintaining regional organ perfusion even under conditions of partial support. This may have important implications for clinical practice in patients with advanced heart failure.
In 10 adult goats (61.3±6.4 kg), a centrifugal LVAD (EVAHEART, Sun Research, Vienna, Austria) was inserted to the coronary sinus. We compared the amount of coronary flow, the oxygen consumption of the native heart (MVO2), and the pressure volume area (PVA) under varied assist rates and circuit-clamped condition (baseline).

**Results:** There were no remarkable changes in mean aortic pressure, mean central venous pressure, total flow, and systemic vascular resistance. Coronary flow, MVO2, and PVA remained unchanged at 50% assist rate compared with the baseline condition. At 100% assist rate, all of these parameters were diminished significantly. (Coronary flow: 121.8±34.6 vs 94.4±12.4 ml/min, p=0.048, MVO2: 0.0529±0.0171 vs 0.0394±0.0161 ml/beat/LV100g, p=0.043, PVA: 1320.3±312.3 vs 637.3±380.9 mmHg/LV100g, p=0.00018, Baseline vs 100% assist rate, respectively). There was an upward trend in coronary vascular resistance by 100% assist (0.561±0.178 vs 0.663±0.234 mmHg/ml/mL, p=0.036, Baseline vs 100% assist rate, respectively).

**Conclusions:** Full bypass by a continuous-flow LVAD can diminish coronary perfusion, possibly due to decreasing oxygen demands of the native heart and the reactive resistance change in the coronary artery due to autoregulatory and reactive changes in the vessel wall. Therefore, further investigation on ischemic heart models is currently in process of preparation.

**OS5 (218)**
IN VIVO TEST OF A NOVEL INFLOW CANNULA FOR LESS INVASIVE AND ANTITHROMBOGENIC LVAD SUPPORT
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**Objectives:** Left ventricular assist device (LVAD) support has been an effective treatment for refractory heart failure, however, there are several problems to solve in order to get better outcome of its use. One of them is related to an inflow cannula that is usually inserted into the LV apex and sometimes causes complications such as LV thrombus and/or stricture of the conduit due to intimal hyperplasia around the tip of the cannula. In this study, we investigated the reason of the complications related to conventional inflow cannulae and developed a novel one.

**Materials and Methods:** Nine adult goats and six calves were installed pulsatile or continuous flow LVAD with conventional inflow cannulae in acute or chronic experiment. We traced intra LV flow using color Doppler and three-dimensional (3D) echo imaging, detected blood stagnation in the LV apex, and sometimes found LV thrombi around the tip of the cannula. Endoscopic observation of the intra LV space after LVAD support sometimes indicated adhesion of the tip of the cannula on the endocardium. Then, we developed a novel inflow cannula which was designed like the shape of the trumpet to reduce blood contacting surface area and blood stagnation in the LV. Moreover it was designed to insert less invasively from the LA appendage and to place at the LV apex without any other assist circulation or cardiac arrest.

**Results:** Three adult goats were easily inserted the novel inflow cannula into the beating LV with a specially designed introducer and installed a continuous flow LVAD. Color Doppler and 3D echo imaging demonstrated the better blood flow pattern in the LV with the novel inflow cannula compared to conventional inflow cannulae, and at autopsy the novel inflow cannula fitted the endocardium well.

**Conclusions:** The novel inflow cannula has a potential to accomplish safer LVAD support than conventional cannulae do.

**LIVER AND VARIOUS TISSUES**

**OS54 (249)**
HEPG2 Cell Encapsulation for Further Liver Implantation
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The liver is an organ with a high ability of regeneration, but some factors such as diseases, drugs or alcoholism may affect it and lead to cirrhosis. Up to now, liver transplant is the only treatment available in the most severe cases and many patients die while waiting for an organ. Several artificial and bioartificial systems are under study, aiming at replacing either detoxication or whole liver functions in an extracorporeal circuit. Such systems are of extreme interest for the patient's support in long term: hepatocyte encapsulated in porous biomaterials could be directly implanted in the patient's liver.
The UTC laboratory has a strong expertise in the area of hepatocyte encapsulation in alginate beads, which led to the design of a fluidized bed bioartificial liver. In the present study, our objectives consist in screening different types of biomaterials to optimize implantation of cells in a cavitary tissue. Cell encapsulation will prevent them from immune rejection and act as a niche in the liver.

In a first step, several materials such as collagen or fibrinogen, proteins often used in hepatocyte cultures, were combined with alginate or directly composed the gel. Beads of different diameters were produced using either a co-axial air flow extruder (homemade design) or a device from Nisco based on vibrations.

In a second step, hepatic cells (human cell line HepG2C3A) were mixed with the most promising biomaterials. The viability of encapsulated cells and their functionalities were compared to those observed in our “basic” alginate beads.

In association with the INSERM group in Montpellier, several configurations will be implanted in a rodent model, in order to reinforce the feasibility of the approach. Specific experiments will be developed to localize the position of the cells hosting beads.

OS5 (286)
IN VITRO EVALUATION OF MAGNETIC RESONANCE IMAGING CONTRAST AGENTS FOR LABELING OF HUMAN LIVER CELLS
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Objectives: Magnetic resonance imaging (MRI) is a promising approach for non-invasive monitoring after liver cell transplantation. Aim of this study was to compare in vitro labeling of human liver cells with clinically approved, nano-sized iron oxide particles (SPION) and micro-sized iron oxide particles (MPIO).

Methods: Primary human hepatocytes were isolated from 9 different patients. Cells were cultivated in adhesion and incubated with dextran-coated SPIONs (Endorem) and polymer-embedded MPIOs. Control groups were native cells and iron-stimulated cells. Iron content was quantified and phantom studies were performed using 3.0 Tesla MRI. Parameters for cellular damage and metabolic activity (AST, ALT, Urea), iron metabolism (Ferritin, Transferrin receptor) and Reactive oxygen species (ROS) formation were investigated over a period of six days.

Results: Incubation with SPION for 16 hours or MPIO for 4 hours produced similar iron load of 22×4 µg iron/cell. Phantom studies revealed stronger signal extinction by MPIO-labeled cells compared to SPION-labeled cells. Labeling with both contrast agents did not cause significant increase of cell damage parameters. SPION-labeling induced activation of iron metabolism and ROS formation similar to stimulation with iron. In contrast, MPIO-labeling had no effects on these parameters.

Conclusions: Our results showed that MPIO enabled effective labeling of human liver cells without negative effects on the cellular iron homeostasis. Attention should be paid on possible iron release and oxidative stress caused by biodegradable contrast agents.

OS6 (219)
MATHEMATICAL MODEL APPLICATION FOR PREDICTIVE CRITERIA IN FULMINANT HEPATITIS
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Objectives: In this study, we examined whether the Molecular Adsorbent Recycling System (MARS) with application of a mathematical model can be used in patients with fulminant hepatitis (FH). From our clinical experience, Retroactive deduction has led to these parameters which determine the timing for LT because in our Department MARS is a routine treatment in all FH patients when we have the correct diagnosis with report of emergency liver. The parameters conclude to a positive prognosis include: Glasgow Coma Scale (GCS) score ≥11, intracranial pressure (ICP) ≤15 mmHg or an improvement of the systolic peak flow of 25−32 cm/s with Doppler ultrasound in the middle cerebral arteries, lactate level <3 mmol/L, tumor necrosis factor-α ≤20 pg/mL, interleukin (IL)-6 ≤30 pg/mL, and a change in hemodynamic instability from hyperkistic to normal kinetic conditions, and so define the timing (and indeed the necessity) of a liver transplant (LT).

Methods: In Intensive Care Unit (ICU) we treated 48 patients with FH (25 female and 23 male) with a mean age 35.3 (range 3-56). Standard Medical Therapy was applied (inotrope drugs; plasma and platelet infusion; diuretics; profilaxic antibiotic therapy, etc). Continuous MARS treatment was carried out on all patients with kit change every 8.8±0.9 hours. Heparin bolus (5 IU/min) + continuous infusion of (0.5 IU/h) (22 patients) or flushing saline solution every 30 minutes (26 inotropic drugs; plasma and platelet infusion; diuretics; profilaxic antibiotic therapy, etc). In 2004 we started working closely with a group of engineers from La Sapienza. Our aim was to optimize MARS treatment, by clinically applying what had already been demonstrated in vitro studies through the application of a mathematical model. This improve albinum function in the patient/circuit ratio. By applying this model, improved clearance of several substances was observed. We were also able to better evaluate future clinical evolution.

Results: Of the 48 patients, 39 of which survived, 22 went to the transplant while 17 have continued extracorporeal method, indicating a positive resolution of the clinical condition avoiding LTx. When we obtain an improvement of parameters indicated between 35 and 45 hours with the MARS treatment, we decide to continue extracorporeal treatment. Nine patients have died.

Conclusions: According to data obtained from our experience, we could propose a new prognostic score using a mathematical model which, we think, should be applied to all toxins to be removed and to a statistically significant number of patients. This could be achieved by using the mathematical model and computer simulations that cover the relationship between the pharmacokinetics of the patient and the ability of MARS to extract toxic substances. The tailoring of the model to the patient can be achieved by using a “history matching” approach: patient data monitored early on during treatment can be used to calibrate the model and predict the plasma toxin level during the rest of the treatment.

OS7 (81)
MEASUREMENT OF LIVER INPUT IMPEDANCES FOR VALIDATION OF A HUMAN HEPATIC CIRCULATION MODEL
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Objectives: Machine perfusion (MP) of donor livers (an alternative preservation method) still faces some problems leading to organ damage. To better understand the fluid dynamics of the perfusion process, we developed an electrical analog model of the human liver vasculature. The aim of this study was to assess the model validity by comparing model-predicted hydraulic input impedances (Z) with Z derived from MP experiments.

Methods: During MP experiments with an isolated porcine liver (weight 600 g) on a Liver workstation (ORIS, Belgium), pressure and flow waveforms were simultaneously measured at the liver blood inlets and outlet. Applying signal analysis, the hepatic arterial (ZHA) and portal venous (ZPV) Z were calculated and compared to model-predicted Z. Additionally, a model parameter study was performed to determine the influence of vessel diameter, stiffness and fluid viscosity.

Results: Patterns of model-predicted and measured ZHA and ZPV (magnitude and phase as function of frequency) showed similar frequency-dependent trends. Absolute magnitude values, however, were higher in the porcine liver, attributable to the fact that human livers (and vessels) are larger. At low frequencies, lower viscosity, lower vessel stiffness and larger diameters corresponded to lower ZHA and ZPV magnitudes. Due to alterations in the relative importance of inertial/compliance effects, less negative PV phases are found for larger vessel diameters and lower viscosities, and HA and PV phase shifts towards lower frequencies for lower stiffness.

Conclusions: Measured ZHA and ZPV patterns are in good qualitative agreement with model-predicted values, suggesting validity of the model to describe global organ behaviour upon MP. Given the sensitivity of Z to changes in model parameters, impedance measurements might be useful to assess fluid dynamic organ functionality prior to transplantation. Note, however, that human MP experiments will eventually be necessary to validate the human model.

OS8 (349)
AUTOLOGOUS TRANSPLANT OF ADIPOSE-DERIVED STROMAL CELLS PREVENT ESOPHAGEAL STRicture AFTER ENDOCoscopic MUCosal RESECTION IN DOG MODEL
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Objectives: Endoscopic mucosal resection (EMR) has been an accepted treatment for early esophageal carcinoma. However, resection of a large amount of the esophageal mucosa often causes postoperative esophageal stricture. The aim of this animal study was to investigate the efficacy of autologous adipose-derived stromal cells (ASCs) on the prevention of stricture formation after EMR in dog.
Effects of Cascade Filtration in Combination with Interferon

Objective: In 40% of patients with chronic hepatitis C, standard therapy is unable to eradicate the virus. Since the response to pharmacological treatment depends on the initial viral load, there is a rationale for reducing this load by means of apheresis depletion of the C virus. Aim of this work was to administer cascade filtration (CF) to non-responder patients affected by hepatitis C (pts) before resuming the pharmacological treatment.

Methods: 10 pts underwent 12 sessions of CF, 3 per week (treated plasma volume/session: 3000 mL). After the first week, therapy with PEG-IFN (1.5 µg/Kg/week) plus Ribavirin (1200 mg/day) was associated.

Results: The viral load was defined before and after each CF session, and at the 1st, 3rd and 6th month. The mean pre-apheresis viral load dropped from 2176 275±3108 997 U/mL at the first session to 1486 726±2091 975 U/mL by the fourth (p<0.001), and 347 500±637 428 U/mL before the last (p<0.001). The mean percentage reduction of the viral load went from a minimum of 29.5% to a maximum of 42%. Early virological response was obtained in 70% of these patients as compared with only 10% in an age- and sex-matched control group consisting of 10 patients.

Conclusions: Efficacious removal of HCV was obtained with CF. The successful reduction in the viral load achieved with apheresis led to a better response to pharmacological treatment in patients previously classified as non-responders.

O61 (43)

Improving the Biocompatibility of Adsorbents for Extracorporeal Blood Purification by Albumin Coating

Objective: In combined membrane/adsorption-based extracorporeal blood purification systems, hydrophobic polymers can be used for the removal of markers of liver failure, such as bilirubin, cholic acids, phenolic substances and aromatic amino acids. However, many of these adsorbents have the unwanted side effect to remove Protein C (PC) and, at a lower amount, fibrinogen.

Results: Since PC is a natural anticoagulant, its removal might lead to clotting in the extracorporeal blood circuit. Aim of this study was to coat a hydrophobic resin in order to reduce the PC adsorption without affecting the adsorption kinetics for toxins of liver failure.

Materials and Methods: Hydrophobic adsorbents with different pore sizes were coated in albumin solutions at 10 °C in a lab-shaker. The albumin-coated adsorbents were characterized and compared to non-coated adsorbents in respect of adsorption of bilirubin, cholic acid, tryptophane, phenol and PC in batch tests. To optimize the coating procedure, we modulated the incubation time as well as the albumin concentration of the coating solution. The coating process is based on hydrophobic interactions between the hydrophobic domains of the resin of the adsorbent and the albumin.

Results: Our results show that for the removal of toxins of liver failure, the optimum adsorbent pore size is in the range of 150-200 nm. However, for biocompatibility reasons, a pore size of 300-400 nm should be preferred. The fibrinogen removal of the adsorbent could be improved by albumin coating. The cost-benefit for the coating procedure was best at an albumin concentration of 5%. Albumin coating was most effective, especially to reduce PC adsorption, when performed over night.

Conclusions: We could considerably improve the biocompatibility of hydrophobic adsorbents by albumin coating without significantly compromising adsorption kinetics for relevant toxins of liver failure. Therefore, albumin coating could be an efficient way to optimize biocompatibility of hydrophobic materials that come in contact with human blood or plasma.

O62 (202)

Prolonged Immunoadsorption (IAS) Further Reduces Proteinuria and Stabilizes Disease Activity in Systemic Lupus Erythematosus (SLE)

Objective: SLE is characterized by pathogenic autoantibodies and immune complexes which can effectively be removed by IAS. IAS caused reduction of proteinuria, disease activity (SIS) and autoantibody levels in highly active SLE with renal involvement if patients were treated up to one year. We now evaluated patients undergoing proloned IAS (>1 – 10 years) for the sustainability of the primary response to IAS and for the number of flares, infections and adverse events.

Methods: Of 20 patients undergoing IAS therapy, 13 patients fulfilled the response criteria and were offered prolonged IAS. These criteria were defined as a 20%–reduction (R20) in at least 2 of the 3 outcome parameters (proteinuria, SIS, and antiDNA levels) compared to the results at 3 months, or at least 50% reduction (R50) in all 3 parameters compared to baseline. We defined the end of observation (EOO), as 10 yrs of IAS therapy or by 2009. During IAS, oral immunosuppression and ACE/ATII-inhibitors were kept constant, steroids were
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065 (235)
PRE-CLINICAL SAFETY AND EFFICACY OF IMMULOC - A NEW LEUKAPHERESIS DEVICE
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Objectives: We hypothesized that patients suffering from inflammatory diseases (inflammatory bowel diseases, rheumatoid arthritis and psoriasis) may profit from the therapeutic depletion of neutrophils and monocytes. Therefore, we designed and constructed a leucapheresis device removing these blood cells during extracorporeal treatment of blood.

Materials and Methods: Here we report on data obtained either in an in vitro or a sheep model model of leukapheresis. After rinsing, 450 mL of freshly donated, human blood (flow rate: 50 ml/min, 5 IU/L heparin or 0.38% citrate, n=5) was recirculated for up to 120 min. Blood cells were counted and up-regulation of CD11b/CD18 on neutrophils was analyzed by flow-cytometry. Vascular access in four sheep was achieved with a double lumen hemodialysis catheter inserted into a jugular vein. Apheresis was carried out using a BM11 monitor (50 mL/min, 15,000 IU heparin).

Results: In vitro at t=60 min WBC was at 43±11%, neutrophils at 29±10%, monocytes at 29±8%, lymphocytes at 80±9%, platelets at 66±9% and red cells at 103±6% of the initial. CD11b/CD18 on neutrophils remaining in circulation was up-regulated (55% of positive control, 100 ng/mL LPS). Leucocyte adhesion and receptor up-regulation was strictly depending on Ca2+. These data were confirmed in sheep (WBC: 38% of initial). All treatments were finished without adverse events and no coagulation problems observed.

Conclusions: In vitro and in vivo leucapheresis device IMMULOC was safe and effective. It should be studied further in clinical trials.

064 (53)
INFLUENCE OF DOUBLE-FILTRATION (DFPP) TREATMENT WITH TRI-THERAPY FOR RELAPSING HEPATITIS C VIRUS (HCV) IN LIVER-TRANSPLANT PATIENTS
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Objectives: In Japan, the addition of DFPP to the usual bi-therapy treatment for HCV, i.e. pegylated interferon (pegIFN) and ribavirin (RBV) has shown greater reduction of virus load in HCV patients with chronic hepatitis. Thus, the influence of 5 DFPP sessions in addition to antiviral therapy was tested on 10 genotype 1b HCV(+) liver-transplant patients with relapsing post-transplant HCV infection not responding to pegIFN/RBV. The primary end-point was the eradication of HCV by 18 months after commencing the tri-therapy.

Methods: DFPP was performed for 5 consecutive days via an extra corporeal circuit: blood was filtered through an OP05W (Asahi Medical) that separated plasma from blood cells, the plasma then passing through a 2nd filter (EC50W, Asahi Medical) removing the HCV. The cleansed plasma and the blood cells were then returned to the patient. A 12-month antiviral therapy pegIFN/RBV plus amantadine was commenced after the 2nd session of DFPP.

Results: Tolerance to DFPP was excellent; no graft rejection occurred during study or follow-up. 2 patients left the study due to serious reactions to pegIFN. The main initial HCV load was >6 log. At 6 months, HCV viremia became negative in 4 patients, decreased in 3 patients (partial responders) and one had no viral response. The partial responder patients received another 5 daily DFPP treatments: 1 patient then became negative for HCV and the other 2 remained stable. At 12 months, 5 patients were HCV RNA negative; however, 3 relapsed within the 2 months after cessation of tri-therapy. At 18 months, 2 patients had achieved a sustained viral response.

Conclusions: Tri-therapy with DFPP provided promising results. However, these results need to be confirmed by further studies that focus on the mechanisms of action of DFPP, as the slight decrease in HCV load due to DFPP (~0.3 log per session) is not likely to be the only cause of these results.

066 (216)
PLASMA EXCHANGE IN ELDERLY PATIENTS
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Objectives: In an extracorporeal blood purification technique was designed for the removal of large molecular weight substances from plasma. The aim of the study was to analyze the database of the University Hospital Centre Zagreb (634 patients, 6237 procedures) for indications and complications in patients aged 65 years or older who were submitted to PE during the period from 1982 to 2007. A total of 50 patients in this age group were submitted to PE. The median age of elderly patients was 69 years (range 65-83). This population underwent 253 episodes of PE. The most common indication for PE (76%) was neurological (e.g. myasthenia gravis and Guillain-Barre syndrome), which was more common than in the entire population (i.e. of all age groups) (60%). The second most common indications were hematological diseases, intoxications, and Goodpasture’s syndrome. 94% of elderly patients showed improvement, compared with 75% of younger patients. Two elderly patients with Guillain-Barre syndrome died, and a patient with pemphigus vulgaris had no change in clinical status. Complications occurred during 11.5% of treatments, compared to 3.9% in the younger group. The most common complications were clotting (3.7%), blood access difficulties (1.5%), mild-to-moderate allergic reactions (1.5%), and precordial oppression (0.6%). PE is rarely used in the elderly population, but when carried out by experienced staff, it is a safe and efficient method that may significantly improve the outcome of elderly patients with appropriate indications.

CARDIAC CLINICAL

066 (237)
USABILITY AND INTUITIVE USAGE OF VENTRICULAR ASSIST DEVICE PERIPHERALS BY UNTRAINED PARAMEDICS
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Objectives: VAD systems are increasingly used, with patients of different age and condition living at home and participating in normal life. In case of emergencies, but also in conjunction with non-VAD-correlated diseases and complications, the assistance or emergency measures by paramedics and laypersons gets important. However, because these systems are yet not included into first-aid, and because of the variety of available systems, an intuitive use is crucial. Aim of this trial was to evaluate the intuitive usability of the existing systems and to elaborate key features for such intuitive use.

Materials and Methods: Various emergency situations (unintended drive-line disconnection, empty batteries, unintended battery-disconnection) known from clinical practice in 2 different VAD-types (Heartware HVAD, Thoratec HeartMate 2) were simulated with a dummy. The untrained and unprepared paramedic personnel (n=8) was randomly assigned to emergency scenarios with the aim to bring an unconscious patient (dummy) back to consciousness by solving the simulated error. Actions and times were analyzed from video-recordings, and a standardized reply form was answered by every person.

Results: 70% (n=62) of the paramedics solved the problem, whereas only 2% (n=2) recognized the error and solved the problem intuitively without any help of a documentation (a special emergency card developed in our centre, and attached to the system). 11% ignored this emergency card completely. 44% (n=40) started cardiac massage although it was neither necessary nor recommendable. 9% (n=8) ignored the alarming controlling unit completely. Numerous details of usability were recorded.

Conclusions: VAD systems should be self-explanatory, with clear marking of components and connectors (colours, labels, signaling). The handling of VADs in emergency and error situations and the training should be standardized.
O67 (264)
REVERSE REMODELLING IN PARTIAL MECHANICAL CIRCULATORY SUPPORT
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Objectives: Full left ventricular (LV) unloading of at least 40 days induces LV reverse structural remodelling, evidenced by normalization of passive end-diastolic pressure-volume relationships (EDPVRs). We sought to investigate the reverse remodelling effect of partial unloading.

Methods: Passive LV EDPVRs were obtained from explanted hearts of 19 patients with chronic heart failure (CHF) undergoing heart transplantation without LV support, 19 CHF patients with full and 5 with partial support before transplantation, and 5 normal human hearts not suitable for transplantation. LV support was at least 40 days. Pressure-volume relationships were measured immediately after unloading of the heart by progressive inflation of a fluid-filled intraventricular balloon with increments of 10 mL, while monitoring the pressure inside the balloon at each increment. LV dilatation was indexed by the volume at which LV pressure reached 30 mmHg.

Results: The volume at which LV pressure reached 30 mmHg was the smallest in healthy hearts (95±12 mL); in patients with partial unloading (196±22 mL; p=0.03, compared to unsupported hearts) it was significantly smaller than that of the chronically failing unsupported hearts (265±60 mL), but larger than the fully supported hearts (150±30 mL; p=0.001, compared to unsupported hearts).

Conclusions: Partial mechanical unloading induces reversal of LV dilatation, but to a lesser extent than full LV support.

O68 (330)
SAFETY AND EFFICACY OF A NOVEL TEMPORARY STERNAL SPREADER IN THE MANAGEMENT OF SEVERE POSTCARDIOTOMY CARDIOMYOPATHY SHOCK: A PRELIMINARY REPORT STUDY
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Objectives: Despite the elevated risk for postoperative infective complications (mediastinitis/sepsis [1-6%]) and acute hemodynamic instability (12-50%) after abrupt delayed sternal closure (DSC), open chest management (OCM) with DSC is a well-known tool to favor myocardial recovery after severe postcardiotomy cardiomyopathy shock (SPCCS). We evaluated the outcome of patients with refractory SPCCS treated with a novel temporary sternal spreader (TSS), allowing myocardial recovery by a progressive hemodynamic-controlled “closed” remote approximation of the sternal edges.

Methods: Five patients with refractory SPCCS showing acute hemodynamic instability at sternal closure, were implanted with the new TSS, consisting of stainless steel branches linked to two diverging plates of polyether ether ketone, whose progressive opening/closing mechanism can be controlled from outside the chest by means of a rotating steel wire; the system is closed by an elastic Esmark latex membrane sutured to the skin edges thus achieving a sterile field. Swan-Ganz monitoring was employed and hospital outcome registered.

Results: The device was successfully implanted in all (100%) patients without complications or failures. Progressive approximation of sternal edges, titrated on cardiac-index values, was successfully completed allowing subsequent uneventful sternal closure in all. Mean-time from SPCCS to sternal closure was 67±14.4 hrs. In no patient developed infective complications or late hemodynamic instability after device removal and sternal closure. One patient (20%) died of MOF on POD9.

Conclusions: Despite the limited number of patients enrolled the new TSS seems safer and more effective than traditional OCM+DSC at allowing complete myocardial recovery after SPCCS, avoiding infective complication and/or hemodynamic instability related to the abrupt sternal closure.

O69 (160)
IS THERE A DIFFERENCE IN ORGAN RECOVERY AFTER FULLVERSUS PARTIAL CIRCULATORY SUPPORT?
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Objectives: Heart failure affects all organ systems, especially kidneys. Recently a rotational micro-pump (Synergy(TM) micro pump, CircuLite® Inc.) has been developed. This assist device delivers partial support. In our institution we have implanted 14 Synergy(TM) micro-pumps. In the same period we implanted 29 HeartMate IIT™ (Thoratec® Corporation) devices. Recovery of organ function under full versus partial circulatory support was analyzed retrospectively.

Methods: All patient files were reviewed at fixed intervals during the first year on support. We compared postoperative data between patients on partial support (Group CL, Synergy(TM) micro-pump) and patients on full support (Group HM, HeartMate IIT™)

Results: Preoperative NT-proBNP levels are markedly elevated in both groups (Group CL: 7854 ng/L; Group HM: 7952 pg/L; p=NS) and decrease significantly after 2 months in both groups (Group CL p<0.05; Group HM p<0.001) but remain elevated. Creatinine levels before operation are elevated in both groups (Group CL: 1.5 mg/dL; Group HM: 1.5 mg/dL; p=NS) and return to normal in both groups after 2 weeks on support (Group CL p<0.05 and Group HM p<0.01). Preoperative bilirubin is elevated in Group HM (2.1 mg/dL) but not in Group CL (1.0 mg/dL). Postoperative bilirubin levels increase after 2 weeks in Group HM (p<0.05) but not in Group CL, and at 2 months bilirubin levels are decreased towards normal in both groups. Baseline CRP levels are elevated in both groups (Group CL: 11.7 mg/L versus Group HM: 7.1 mg/L; p<0.05). In the immediate postoperative phase (day 1 to 10) CRP levels increase further in both groups with a peak value at day 3. In Group CL the level drops faster but remains slightly higher compared to the preoperative value whereas in Group HM the CRP level decreases significantly after 2 months compared to preoperative levels (p<0.05).

Conclusions: Heart failure affects all organs, especially kidneys. Both full and partial support improves kidney function postoperatively. This might be in particular interest for partial support where better renal function might allow ACE-inhibitors to be up-titrated.

O70 (77)
USING EXTRACORPOREAL LIFE SUPPORT TO RESUSCITATE ADULT POSTCARDIOTOMY CARDIOMYOPATHY SHOCK: TREATMENT STRATEGIES AND PREDICTORS OF SHORT-TERM AND MID-TERM SURVIVAL
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Objectives: Postcardiomyopathy extracorporeal life support (ECLS) is a resource-demanding therapy with variable results among institutions. We aimed to develop an organized protocol of postcardiomyopathy ECLS to improve the outcome.

Methods and Results: From January 2003 to June 2009, a total of 110 patients who received ECLS because of postcardiomyopathy shock were enrolled in this retrospective study. The preoperative, perioperative, and postoperative variables, including assessments of the European System for Cardiac Operative Risk Evaluation (EuroSCORE) and ECLS-related organ injuries, were analyzed for mortalities in hospital and after hospital discharge. Mean age (year), preoperative additive EuroSCORE, and left ventricular ejection fraction (LVEF) of the studied patients were 60(±14), 9(±6), and 43%(±20%). Sixty-seven patients were weaned from ECLS and 46 had hospital discharge. The mean length of ECLS was 143 h (±112 h). Multivariate analysis revealed that an age > 60 years, the necessity of continuous arteriovenous hemofiltration, a maximal serum total bilirubin > 6 mg/dL, and an ECLS > 110 h were independent predictors of inhospital mortality in adult patients with postcardiomyopathy ECLS. In addition, a persistent heart failure status (LVEF < 30%) was the independent predictor of mortality after hospital discharge. A risk-predicting score of in-hospital mortality of postcardiomyopathy ECLS was also developed for clinical application.

Conclusions: On the basis of the above-mentioned findings, a comprehensive protocol of postcardiomyopathy ECLS was designed with the primary target (achieving adequate hemodynamics within the first 24 h), secondary target (maintaining the coagulation demand and decision within 7 days), and tertiary target (follow-up after hospital discharge).

O71 (208)
PRIMARY TRANSRADIAL CORONARY INTERVENTIONS IN ACUTE MYOCARDIAL INFARCTION
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Primary coronary interventions (PCI) performed by the transradial approach (TRA) in patients with acute myocardial infarction are more challenging compared to the classical interventions through transfemoral approach (TFA). In order to evaluate the results of TRA in STEMI patients, we have analyzed the data from the interventional cardiology registry at the University Cardiology Clinic in Skopje, Macedonia.

In the period from 1 January 2007 to 3 March 2009, we prospectively analyzed 1712 consecutive patients, in which coronary angiography and primary PCI were performed within the first 24 hours from the first STEMI symptoms. Patients were separated in two groups: TRA group...
(810/47.3%) and TFA group (802/52.7%). We have analyzed the following data: the success of arterial approach, procedure time intervals, intervention final result success, hemorrhagic complications and mortality rate. We also followed the acceptance rate of TRA approach by the operators as a first choice interventional approach.

There were no significant basic clinical differences between the groups. 80.5% were men, with mean age 58 years (+/-10.5 years) and 19% were diabetic patients. There was no statistical difference between the groups in the procedure duration (43/45 min), reperfusion time (13 min), radiation time (7.9 min), culprit infarction artery (LAD 48%; RCA 38%; LCx 14%) and intervention success (99%). Hemorrhagic complications at puncture site in TRA interventions group were significantly rare (1.8% vs. 3.6%; p<0.01), with almost twice lower mortality in TRA group comparing to TFA group (TRA 4.4% vs. TFA 7.8% - OR 1.8 95% CI). In the follow-up period the percentage of TRA interventions continuously raise from 23% in 2007 to 34% in 2008, 60% in 2009 and 92% in the first quarter of 2010.

Primary transradial coronary interventions have successfully taken the place of transfemoral approach in the treatment of patients with acute myocardial infarction in Macedonia.

**LIVER WORKING GROUP**

**072 (173)**

**THREE SHORT-TIME NONSURVIVAL EXPERIMENTAL SURGICAL MODELS OF ACUTE LIVER FAILURE – FEASIBILITY AND APPLICATIONS**

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**Objectives:** In the last decades many different toxic and surgical models of acute liver failure (ALF) have been developed. However a large animal model with full pathophysiological relevance appropriate for testing artificial and bioartificial liver support systems, as well as for hepatocyte transplantation, still does not exist. The aim of this study was to evaluate three different surgical models of ALF with regard to their pathophysiological relevance and applicability in testing new liver support therapies.

**Methods:** Thirty Czech landrace female pigs were included into the study. The animals were divided into 4 groups, ischemic model with portocaval anastomosis (PCA), nonanatomical 70% liver resection (RES) and hepatectomy with Y-prothesis replacement (HEP) were performed in 8 pigs each. The ALF onset was confirmed by comparison to 6 pigs in control group (CON). The experiment time was set up to 12 hours and the animals were not woken till euthanasia. Hemodynamic parameters and intracranial pressure (ICP) were measured continuously and biochemical and blood gas analysis every three hours. Coagulopathy (INR >1.5) and intracranial hypertension (ICP > 20mmHg) were considered as criteria of ALF.

**Results:** Animals in interventional groups developed significant (p<0.05) increase of ICP, ammonia and bilirubin from 6 hours comparing to control group. Prothrombin time (INR) raised slowly and reached the level of 1.5 in 12 hours in PCA and RES groups. The criteria of intracranial hypertension (IH) were fulfilled first by PCA group (7 hour), then by HEP (10 hour) and RES groups (12 hour). High doses of noradrenalin were needed to maintain mean arterial pressure above 60mmHg in all animals in the last 3 hours of experiment.

**Conclusions:** All presented models fulfilled criteria of IH accompanied by coagulopathy in resection and ischemic models. We confirmed ischemic model as the most reliable with relative short therapeutic window as compared with resection model. The anhepatic model was the easiest to perform.

**073 (287)**

**TEMPORAL MICRORNA GENE EXPRESSION PROFILES OF THE REGENERATING RAT LIVER AFTER PARTIAL HEPATECTOMY**

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**Objectives:** The liver has the unique capacity to regenerate after surgical resection. However, the regulation of liver regeneration is incompletely understood and cell expansion in vitro remains difficult. Recent reports indicate a critical role for small non-coding microRNAs (miRNAs) in the regulation of proliferation and cellular function by binding to target miRNAs, which subsequently leads to translational arrest.

**Methods:** We hypothesized that miRNAs mediate fine-tuning of liver regeneration and performed microarray analyses of miRNA expression during the proliferative phase after 70% partial hepatectomy in rats (n=3). Putative targets of differentially expressed miRNAs (>2.0 fold) were determined using a bioinformatic approach. Standard parameters of liver regeneration (BrDU, IL-6, HGF) were applied to characterize the temporal pattern of liver regeneration.

**Results:** Nine miRNAs were expressed at significantly lower levels 12-48 hours after resection including three members of the let-7 family, while none was expressed at significantly higher levels. MIRNA downregulation correlated with the activity of hepatic regeneration which peaked at 24 hours after resection. Interestingly, many molecules involved in cell cycle regulation, apoptosis and carcinogenesis were identified among the predicted targets. Consistently, cyclin D1 expression was increased as determined by Western Blot analysis.

**Conclusions:** Our results indicate that miRNAs might play a critical role in regulating liver regeneration, likely by fine-tuning cell cycle control in a temporal pattern. Current experiments will elucidate the molecular pathways involved in miRNA-based control of liver regeneration.

**074 (345)**

**NECROSIS AND REGENERATION IN EX Vivo PERFUSED PORCINE LIVER**

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**Objectives:** Ex vivo perfused porcine livers have been used for temporary support during acute liver failures. The aim of this experimental study was to assess the histological changes that occur and to correlate these with factors that may influence.

**Methods:** Five porcine livers were harvested, preserved in cold ice and reperfused for six hours in an extracorporeal circuit using autologous normothermic blood. Tissue biopsies were collected hourly. The ISHAK score was used to quantify hepatic necrosis and immunohistochemistry to evaluate apoptosis and regeneration. Liver weight, perfusion parameters, arterial blood gases and blood samples were also collected.

**Results:** The ISHAK score peaked immediately before reperfusion and 4 hours after the start. Scattered necrosis, microvesicular steatotic vaculization, sinusoidal dilatation and red cell extravasation were present. Anion gap acidosis was associated with the score. An inverse correlation was present between the score and the regeneration, and between this and the liver weight. No significant changes were observed for the apoptotic index.

**Conclusions:** The ex vivo perfused liver model showed that hepatic necrosis is present during extracorporeal liver perfusions, follows a definite pattern and is inversely correlated with regeneration. Apoptosis is not increased over the baseline levels.

**075 (228)**

**3D HEPATIC CELL CULTURE FOR VIROLOGY STUDIES**

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**Objectives:** Worldwide, approximately 170 million individuals are infected with hepatitis C virus (HCV), an enveloped positive-strand RNA virus that replicates in the hepatocytes. Since the discovery of HCV, researchers have encountered difficulties with in vitro and in vivo models. The 2D environment of plastic substrates may alter gene expression and prevent cellular differentiation. As an alternative, we propose to use the fluidized bed bioreactor for hepatic cell cultured in alginate beads, so as to promote HCV permissiveness and viral production.

**Methods:** This work investigates an application of the alginate encapsulation technology to culture human hepatoma cells (Huh-7.5.1) in a bioreactor. In the present paper, we focused on the feasibility of cell encapsulation in a structure allowing bi-directional transfers of viruses. The growth kinetics of Huh-7.5.1 cells and their viability in alginate beads were followed by Alamär Blue test, in dynamic versus static conditions. The alginate matrix porosity is determined applying microscopic approach, and quantified by diffusion test.

**Results:** The alginate encapsulation achieves an increase in the cell density in the culture, in comparison to the 2D conditions, opening the HCV potential accessibility to cells. Manipulations of different encapsulation conditions, particularly of the initial alginate concentration, allow the control over the matrix
porosity to study the proliferation and growth of encapsulated Huh-7.5.1 within the bead as well as the viral diffusion to the bead.  

**Conclusions:** 3D cultured Huh-7.5.1 cells may represent a more appropriate physiologically relevant system for further in vitro virology studies. The fluid dynamics of the 3D culture system may promote in vivo-like exchange of efficient cell-to-cell interactions, providing an opportunity to study HCV entry and the effects of HCV infection on host cell function.  

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**O76 (G13)**

**3D BIOREACTOR CULTIVATION OF THE HUMAN HEPATOMA CELL LINE HEPG2 AS A PROMISING TOOL FOR IN VITRO SUBSTANCE TESTING**


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**Objectives:** New challenges in drug development and drug testing arise from regulatory requirements. Animal trials have to be replaced by cell culture assays, preferably by test systems with human cells. Standard 2D monolayer cultures are often unsatisfactory and therefore tissue-like 3D cultures are suggested as an alternative. In this study in vitro assessment of hepatocyte xenobiotic metabolism was performed in a flow chamber and a miniaturized fixed bed bioreactor.

**Methods:** Cultivation of HepG2 cells was carried out in static culture (12-well-plate) or dynamically in a flow chamber and a fixed bed bioreactor. Static and dynamic cultures of hepatoma cells (HepG2) were compared for their xenobiotic metabolising capacity determined by measuring the EROD (Cytochrome P450 1A1 and 1A2) activity. Induction of Cytochrome P450 1A1 and 1A2 activity was carried out using 3-Methylcholanthrene.

**Results:** Bioreactor cultivation of HepG2 cells was carried out for 2 weeks. Staining with acridine orange and propidium iodide revealed cell clusters of viable cells on the ceramic carriers (Sponceram, Zellwerk) in the fixed bed reactor and on polymeric meshes (Fibracel, New Brunswick Scientific) in the flow chamber. Cell specific activities in functional assays were dependent on type of carrier, time point and cultivation system.

**Conclusions:** The cell line HepG2, although not optimal regarding CYP expression levels, can be used as a valuable reference system for further studies in miniaturized bioreactor systems. Extended cultivation on 3D carrier systems, especially in perfused systems, is feasible and appropriate for long-term studies of hepatocyte xenobiotic metabolism.

**Tissue engineering technologies**

**O77 (G25)**

**CAN TRANSMEMBRANE IMPEDANCE BE A MARKER OF ENDOTHELIAL CELLS GROWTH IN CULTURES ON HOLLOW FIBER MEMBRANES? PRELIMINARY STUDY**

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**Introduction:** Monitoring of cells growth in the culture is very important. It is difficult to perform when cells are cultured on the inner surface of capillaries of the hollow fiber bioreactor. The cells seeded onto a surface of membrane give additional resistance to alternating current flow through the membrane.

**Objectives:** The main objective of this preliminary study was to investigate whether the transmembrane impedance may be a marker of endothelial cells growth in cultures on hollow fiber membranes in bioreactor.

**Methods:** The modulus of transmembrane impedance (MTI) (between the inner and outer compartment) was measured at 12 frequencies (10Hz-50kHz) during 48 hours at 10-minute intervals. In the first part (0-24 hours) of the experiment HepG2 cells (Human Umbilical Vein Endothelial Cells) were seeded on the inner surface of bioreactor capillaries and cultured. At the end of the experiment (first part), cells were removed from bioreactor using trypsin solution. The number of removed cells was evaluated. In the second part (24-48h) of experiment the MTI was monitored without cells.

**Results:** The average value (N=130) of MTI from the second part of experiment at all frequencies was lower than the average value (N=141) from the first part (p<0.0001). The maximal MTI changes were from -41.4% to -43.0% in the frequency range 500Hz – 20kHz.

**Conclusions:** MTI after cell removing remarkably lowers, so it seems to be a good marker of cells occurrence on the inner part of capillary membranes in bioreactor.

**O78 (G06)**

**A NOVEL 3-DIMENSIONAL MIGRATION ASSAY FOR HIGH-THROUGHPUT SCREENING (HTS)**


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**Cell migration is a critical and central process in the development and maintenance of living organisms. Cells in the body will often move in a particular direction to a specific location to complete their functions. Migration is a cyclical process in which a cell extends protrusions at its front and retracts its trailing end. Cells in tissues mainly migrate in response to specific external signals. The process of cell migration is important in such things as embryonic development, wound repair, differentiation and immune responses. Cell migration contributes to pathologies including vascular disease, chronic inflammatory diseases and tumor formation. Therefore, the search for components promoting or inhibiting cell migration are therapeutically promising.**

**We have established novel 3D migration assays where an organotypic skin model is used for standardized and automated analysis of wound healing or tumor spread. The methods mimicking substances in high-throughput screening systems are able to distinguish between the effects of various stimulants and inhibitors of cell migration.**

**1. The Electric Cell-Substrate Impedance Sensoing (ECIS, invented by B. Lauga and C. Keese) was adapted, allowing migration, proliferation and differentiation of keratinocytes over a 3D matrix (collagen 1 or fibrin-clot) with high temporal resolution and without the addition of any markers.**

**2. Our Cell Migration Assay from Platypus Technologies, Madison, WI. This newly established 3D skin models migration assay provide an ideal tool for the detection of the phases of skin repair in high-throughput screening, resulting in faster, cheaper and more physiological screening of bioactive agents.**

**These newly established Cell-based Test Systems are ideal techniques for the screening of potential bioactive substances and offer an innovative and novel way to discover new therapeutic drugs which will prove invaluable to the pharmaceutical industry.**

**O79 (G26)**

**COOPERATION OF DERIVATIZED POLYSACCHARIDES AND GROWTH FACTORS AND THEIR EFFECTS ON THE PROLIFERATION AND DIFFERENTIATION OF DIFFERENT CELLS**

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**Objectives:** Glycosaminoglycans (GAG) are part of the extracellular matrix and regulate the activity of growth factors. In tissue engineering GAG analogous substances, synthesized by chemical modification of polysaccharides, are used for that purpose. Here, we present that modified cellulose and chitosan with specific substitution degree are superior to naturally occurring GAG such as heparin.

**Methods:** Cellulose and Chitosan were sulfated and a part of the celluloses was subsequently carboxylated by oxidation or carboxymethylated. The binding to growth factors was examined with a competitive assay using heparin-agarose beads. The influence on the proliferation of 3T3 fibroblasts was investigated in the presence or absence of basic fibroblast growth factor (b-FGF). The effect on osteogenic markers was examined in with of bone morphogenetic protein-2 (BMP-2) in murine C2C12 myoblasts and the formation of extracellular matrix was measured with the derivatives and transforming growth factor-β1 in human mesenchymal stem cells.

**Results:** Maximum sulfation degrees of 1.94 and 1.77 could be reached for cellulose and chitosan, respectively. The oxidation took only place at O-6 position and carboxymethylation was found at O-2, O-3 and O-6 of cellulose.

**Conclusion:** Enhanced proliferation of 3T3 cells could be observed. The strongest effect on the type of growth factor. The b-FGF induced proliferation was strongly enhanced by sulfated celluloses. Moreover, even without b-FGF, a significant enhanced proliferation of 3T3 cells could be observed. The strongest effect on osteogenic activity was found for highly sulfated chitosan.

**Conclusions:** Sulfated and carboxylated celluloses derivatives represent highly effective alternatives to GAG to modulate growth and differentiation of primary and adult stem cells.
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O80 (307)  
CONTROLLED PERFUSION ENHANCES SOLUTE TRANSFER TO THE SHELL OF HFMBs FOR BONE TE  

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Objectives: Development of engineered grafts to repair large bone defects is limited by O2 and nutrients supply to cells in large 3D scaffolds, which may compromise cell viability. Hollow fibre membrane bioreactors (HFMBs) provide a distributed source of nutrients and oxygen supply to cells in large 3D scaffolds. In this work the effect of perfusion flows on solute transfer to extracapillary space (ECS) of HFMBs was studied with tracer experiments.

Methods: The HFMBs used consist of a bundle of microporous HFMs fitted in an acrylic housing with medium flowing in the intracapillary space (ICS). Dimensional analysis of solute transport equations in ICS and ECS suggest that transfer is mainly determined by the non-dimensional membrane pressure modulus alpha and inlet radial Pelet number Per. Operating conditions were set to promote transfer to ECS: by diffusion, under low to high Starling flows, or by pulsatile radial perfusion. HFMBs were challenged with an inert tracer concentration step, and HFMB cumulative residence time distribution and mean residence time were estimated. A model consisting of two CSRTs with interchange with a dead volume was used to analyze data. Magnitude of maximal Starling flows in HFMBs with closed shell was correlated to ECS volume transfer. HFMBs were operated in mass transfer mode (MVTM) from the ICS as estimated from model parameters. Tracer concentration was also measured in ECS in time after challenging the HFMB.

Results: Matter in the ICS was well mixed and distribution well described by one CSTR. Transfer alpha from 0.2 to 0.6, and Per from 0.038 to 3.6 made transfer shift from diffusive to convective yielding maximal Starling flows from 1.3x10^{-3} to 1.3x10^{-1} ml/min. Correspondingly, VMTM increased from 20% to 66% to 100% of ECS volume and tracer concentration in ECS increased faster, and being fastest under pulsatile radial perfusion.

Conclusions: Results suggest that controlled perfusion in HFMBs enhances solute transfer to the ECS and might enable bone cell culture in large TE constructs.

O81 (189)  
IN VITRO DIFFERENTIATION OF STEM CELLS FOR THE REGENERATION OF INTERVERTEBRAL DISC USING APPLICATION OF TISSUE-ENGINEERED HUMAN INTERVERTEBRAL DISC (IVD)  

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Objectives: Intervertebral disc (IVD) degeneration is still a problematic musculoskeletal disorder. We developed recently a new tissue engineered approach for the IVD regeneration, based on the 3D culture of autologous human IVD cells in a polyurethane (PU)-fibrin composite. To implement such applications in the clinical routine, a method is needed to standardize the processes and reduce the costs. Therefore an automated system was developed in order to isolate, propagate and characterize IVD cells in large scale for the PU-fibrin constructs production.

Methods: IVD biopsies were obtained from surgeries on low back pain patients. Cell isolation was either performed manually using standard or automated procedures. For automated processing, a liquid handling robot based on the Tecan® Freedom EVO®, combined with a tissue dissociation tool and a cell detection platform, was applied.

Results: Automated instrument: Cell yield and viability by manually and automated processed cells were very similar. Regular automated cell confluence measurement allowed the in situ investigation of the cell growth kinetic, similar by manual and automated processed cells, and permitted decisions on cell splitting in an automated way. Specific marker expression of manually and automated processed cells, analysable by automated immuno-fluorescence staining and detection, was very similar. Cell-based construct: Expanded IVD cells, isolated from pathoimaging, reacquired the capability to synthesize extracellular matrix molecules by cultivation in PU-fibrin composite, indicating the potential of the therapeutic approach.

Conclusions: The presented automated procedure will allow the implementation of such approaches using autologous cells and scaffolds like PU-fibrin structure into the clinical routine.
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**Objectives**: Hemodialysis (HD) patients may be at greater risk of cardiac arrhythmias and sudden death in post-HD period because of the increased QT interval dispersion on electrocardiograms (ECG). Aim of this study was to compare post-HD recorded QT interval duration in various groups of HD patients according to the presence of arterial calcifications (AC).

**Methods**: In a cross-sectional study we examined 109 HD patients (63 male; mean age 51.3±23.1 years; HD duration 95.2±44.3 months). Primarily, we evaluated the presence of arterial intima (AI) and arterial media calcifications (AMC) using plain radiography of the pelvis. Then we compared the QT interval duration calculated from the post-HD recorded 12-lead ECG among the groups of patients with different type of AC.

**Results**: The patients without AC had significantly shorter QT (346.2±23.1 ms vs 381.2±24.3 and 378.6±25.7 ms, p<0.01) and corrected QT (QTc) interval (409.2±21.8 ms vs 463.5±22.3 and 458.4±20.8 ms, p<0.005) duration in comparison with the patients with AI and AMC presence on their radiograms. There were no significant differences in QT and QTc interval duration between the patients with AI and AMC. Multivariate adjusted logistic regression analyses (with group of the patients without AC as the reference value) did not identify QT and QTc intervals dispersion as factors independently and significantly associated with the appearance of both AI and AMC in our HD patients.

**Conclusions**: Post-HD recorded QT/QTc interval is prolonged in HD patients with AI and AMC presence on their radiograms of the pelvis. According to this we can conclude that those patients are prone to cardiac arrhythmias and sudden death.

**O85 (50)**

**ADDITIONAL DISTURBANCES OF FLOW PATTERNS WITHIN THE VENOUS ANASTOMOSIS CAUSED BY SINGLE-NEEDLE HEMODIALYSIS**

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**Objectives**: Single-needle dialysis is a therapeutic option used especially in cases where blood removal and return are only possible via one cannula. Most of the papers regarding the single-needle hemodialysis investigated the efficiency of this method compared to double-needle dialysis. However, the impact of single-needle therapy on hemodynamics within the venous anastomosis of the arteriovenous access is unknown. Especially, if prosthetic graft material is used for vascular access, blood flow rates of more than 700 ml/min are required to prevent thrombosis. High flow rates, however, lead to increased shear stress at the venous wall. Therefore, the additional and oscillating stress caused by the single-needle technique was investigated.

**Methods**: We investigated the standard form of a venous end-to-side anastomosis by Computational Fluid Dynamics (ANSYS 12.1, ANSYS Ltd., USA). Pulsatile mass flow profile at Reynolds numbers of 1620 (systolic) and 740 (mean) was specified as the inlet boundary condition. The mean flow rate of the single-needle dialysis machine was set to 200 ml/min, i.e. flow rates of ~400 ml/min within the withdrawal phase and ~400 ml/min during the return phase.

**Results**: In the return phase, the wall shear stress at the venous wall is four times higher than in the withdrawal phase (40 Pa vs. 10 Pa). Another feature we observed is the turning back of the flow in the anastomosis during the late diastolic period in the withdrawal phase.

**Conclusions**: The long axis of the vascular endothelial cell is usually oriented parallel to the direction of the blood flow. Cells become flat and elongated under increased shear stress. In regions with low shear stress, cells are short and rounded, and gaps can be found between them. Furthermore the demonstrated oscillating flow in the anastomosis may cause the endothelial cells to change their orientation and shape during every cardiac cycle. Thus, the single-needle technique promotes the development of intimal hyperplasia at the venous anastomosis.

**O86 (215)**

**EFFECT OF LDL APHERESIS ON CIRCULATING ENDOTHELIAL PROGENITOR CELLS IN FAMILIAL HYPERCHOLESTEROLEMIA**

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**Objectives**: Long-term treatment with LDL-apheresis (LA) has been shown to reduce the incidence of cardiovascular events in patients affected by familial hypercholesterolemia (FH). Data from experimental studies suggest that circulating endothelial progenitor cells (EPCs) can repair the vascular lesions caused by atherosclerosis. Since a reduction of these cells has been demonstrated to predict atherosclerosis progression, aim of this study was to verify whether LA can increase the percentage of EPCs.

**Methods**: In 15 patients affected by FH in periodical treatment with LA, the percentage of EPCs was determined before and after performing LA, and compared with the values in 15 control subjects and 15 hypercholesterolemic patients treated with statins.

**Results**: Significant differences were found in FH patients between the pre-apheresis percentages of CD34+/KDR+, defined as EPCs by a wide consensus of opinion, and the values found 24 h after the procedures (0.00868±0.003 vs 0.01090±0.002%, p<0.005). Instead, the percentages of CD34+/KDR+/CD133+, considered as an immature subset of EPCs, remained substantially unchanged. In any case, a significant reduction in the percentage of EPCs was observed in both patient groups as compared to controls, at all the assessment times.

**Conclusions**: In the short term LA seems to stimulate mobilization of CD34+/KDR+ cells. Hypercholesterolemic patients show a lower percentage of EPCs than controls. There were no differences in the EPCs percentages between the two patient groups, despite the fact that the LDL cholesterol levels were higher in the group undergoing LA.

**O88 (119)**

**A NEW METHOD FOR PULSE PULSE/PULL HEMODIALYSIS; ANIMAL EXPERIMENTS**

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Many trials have been conducted to increase mid-sized molecular removal in maintenance hemodialysis. Even though hemodialfiltration is assumed as a gold standard for higher convective therapy, alternate repetition of forward and backward filtration during hemodialysis increases the total filtration volume and consequent convective mass transfer. A new method to enhance these both internal filtration and backfiltration was devised. The devised method is based on utilizing pulsatile pumps in dialysate stream
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O90 (32)

LIPOPROTEIN LIPOASE RESPONDS SIMILARLY TO TINzapARIN AS TO CONVENTIONAL HEPARIN DURING HEMODIALYSIS


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In recent years there has been a shift to low molecular weight (LMW) heparin preparations for anticoagulation during hemodialysis (HD). Studies in experimental animals have shown that LMW-heparin releases lipoprotein lipase (LPL) as efficiently as unfractionated (UF) heparin, but are less able to retard hepatic uptake of the lipase. This raises a concern that the LPL system may become exhausted by LMW-heparin in patients on HD. We have explored this in the setting of clinical HD. Twenty patients were switched from a primed infusion of UF-heparin to a single bolus of tinzaparin (a commonly used LMW heparin). LPL activity in blood was higher on tinzaparin early (40 min) but lower late (180 min) during HD. These values did not change during the 6 month study period and there were highly significant correlations between the LPL activities in individual patients at the beginning and end of the 6 month study period and the activities on UF-heparin and on tinzaparin, indicating that tissue LPL was not being exhausted. High responders remained high and low responders remained low throughout the study period and irrespective of the type of heparin administered. Plasma lipid/lipoprotein levels did not change during the 6 month study period. On repeated measurements over about 1 year before and 2 years after the switch from UF-heparin to tinzaparin, the largest mean change was not a significant 9.8±8.0% decrease in LDL cholesterol.

O91 (234)

HEMODYNAMIC EFFECTS OF VARYING IAB INFLATION DURATION IN VIVO

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Objectives: A detailed description of the effects of varying the timing of inflation and deflation on the hemodynamic parameters associated with Intra-Aortic balloon (IAB) operation is lacking. The study aimed at evaluating the effects of varying the inflation duration on the hemodynamic benefits of IAB.

Materials and Methods: 6 anesthetized open chested sheep were studied. Conventional IAB inflation and deflation timing (CC) was sequentially varied by late inflation (LC), late deflation (CL), early inflation (EC) and early deflation (CE). Left ventricular (Plv), ascending aorta (Pao) pressures and coronary (Qcor) flow were sampled simultaneously at 500 Hz. The measured data were used to determine the hemodynamic benefits of inflation; mean diastolic Pao (MDP), diastolic Qcor (DQcor), and those of deflation; end diastolic Pao (EDP) and mean systolic Piv (SPiv). Hemodynamic parameters of the 5 interventions at 1:1 and 1:3 support frequencies were compared to control (balloon off) using unpaired t-test, and p < 0.05 was considered statistically significant.

Results: Inflation: The greatest increase of MDP was seen at 1:1 CC (91±14 vs. 76±10 mmHg, p<0.01) and at 1:3 CL (85±15 vs. 64±9 mmHg, p<0.01). Highest increase of DQcor was seen at 1:1 CC (196±37 vs. 149±25 ml/min, p<0.005) and at 1:3 CL (190±22 vs. 150±25 ml/min, p<0.001).

Deflation: The greatest decrease in EDP was seen at 1:1 CE (58±5 vs. 69±6 mmHg, p<0.05) and at 1:3 LC (54±12 vs. 58±10 mmHg, NS). Greatest decrease in SPiv was seen at 1:1 CL (67±11 vs. 76±10 mmHg, p<0.005) and at 1:3 EC (65±11 vs. 67±11 mmHg, NS).

Conclusions: Conventional and longer duration of inflation (CC, CL) provide the largest benefits associated with IAB inflation. However, shorter durations (CE, LC) provide the largest decrease in EDP, which is the main benefit of balloon deflation. This indicates the main benefits of inflation and deflation may not be obtained simultaneously, and the duration of inflation could be adjusted discriminatorily based on need; increase of Qcor or decrease of EDP.

O92 (191)

APPLICATION OF A HYBRID MODEL TO IAPB INVESTIGATION

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Objectives: This work aims at developing a hybrid circulatory model that can be used to investigate the hemodynamics and energetics of the IAPB, whose effects were evaluated as a function of selected ventricular variables.

Materials and Methods: The hybrid model is based on merging a computational and hydraulic models. The lumped parameter computational model consists of left and right hearts, systemic, pulmonary and coronary circulation. The hydraulic model presented part of the systemic arterial circulation, essentially a silicon rubber tube representing the aorta which contains a 40cc IAB. Endocardial viability ratio (EVR), Cardiac Output (CO), end systolic (Ves) and end diastolic (Ved) volumes and coronary blood flow (CBF) were analyzed against ranges of left ventricular Emax (0.3-0.5-1.0 mmHg.cm-3) and stiffness VS (30-60 cm3.mmHg-1). All experiments were conducted comparing the selected variables before and after IAPB start.

Results: Changes in ventricular parameters influence the considered variables. In general, IAPB assistance produces lower percentage changes in the selected variables increasing Emax from 0.3 to 1 mmHg.cm-3. The changes in VS from 30 to 60 cm3.mmHg-1 strongly influence both absolute value of EVR and its variations after IAPB onset (97% and 58%, respectively). Ved and Ves changes are rather small but higher for lower Emax and higher VS (47.5% and 8% respectively).
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Conclusions: The developed hybrid model provides a platform to produce stable and repeatable circulatory conditions. Since the physical device (IABP) is connected to the computational model of the circulatory system, it is possible to evaluate the effect of changing any parameter of the model on the physical device. Specifically, the simulation shows that IABP performance is strictly dependent on left ventricular filling and ejection characteristics. Further work is required to study the conditions for heart recovery modelling baroreceptor controls and physiological feedbacks.

CRYOPRESERVATION

O93 (250)
COMPATIBLE SOLUTES REDUCE DMSO CONCENTRATION WITHIN CRYOPRESERVATION OF MAMMALIAN CELLS
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Objectives: Cryopreservation plays an important role in the long-term storage of cells and tissues. A high survival rate is a function of optimal cooling rate, appropriate cryoprotective agent (CPA) and its adjusted concentration. Obstacles for the cryopreservation of 3D samples are inhomogeneous distribution of temperature and CPA due to slow heat and mass transfer. The toxicity of the widely used CPA, DMSO and glycerol, further limits their diffusion into 3D tissue due to restricted equilibration time, also additional processes are necessary to remove the CPA after thawing. Thus, we have investigated Compatible Solutes (CS) L-proline hydroxyectoine (HE) and ectoine as potential CPAs, since they protect cells from stress damage.

Methods: Human pulmonary microvascular endothelial cells (HPMEC-ST1.6R) were used for the experiments. Cells were frozen in 1.8 mL Nalgene® cryovials (1.5 ml, 4.5x10^5 cells/mL) with optimal cooling rate (gained from previous study). Different concentrations of L-proline (5Mm to 100Mm), ectoine (10Mm to 500Mm) and HE (10Mm to 200Mm) were studied in combination with DMSO (0 to 10% v/v). Cells were frozen either directly with freezing medium (FM) containing CS with a 10 min equilibration period or after 48 h incubation in a culture medium (CM) containing CS. Cells that were attached to the culture flask surface after 24 h recultivation were considered as viable cells, the survival rate is normalized to the positive control (fresh cells incubated for 24h).

Results: With 1% DMSO and moderate concentration of CS in FM, cell survival rate could reach 60% (control 20%) comparable to that of 10% DMSO, with 7.5% DMSO cells attached could even be more than that of positive control. Conclusions: Compatible solutes could be used to reduce the concentration of DMSO, however their concentration are restricted due to effect of osmolarity on cells.

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