ABSTRACT BOOK

ORAL PRESENTATIONS

OP 01

Withdrawn

OP 02

ASSESSING THE QUALITY OF DONOR KIDNEYS DURING NORMOTHERMIC MACHINE PERFUSION USING NANOPARTICLE-BASED VITALITY SENSORS

Jesper Staulund[1], Ping Song[2], Haiyun Qi[3], Lars Thrane[4], Pernille Duedahl[5], Thomas Weiss[5], Cyril Moers[6], Anna Krarup Keller[7], Rikke Nørregaard[8], Michael Pedersen[4], Henri Leuvenink[6], Jørgen Kjems[2], Bente Jespersen[5], Marco Eijken[5]

[1]Aarhus University Hospital, Urology and Nephrology, Aarhus, Denmark, [2]Aarhus University, The interdisciplinary nanoscience center, Aarhus, Denmark, [3]Randers Centralsygehus, Randers, Denmark, [4]Aarhus University Hospital, Department of Clinical Medicine - Comparative Medicine Lab, Aarhus, Denmark, [5]Aarhus University Hospital, Nephrology, Aarhus, Denmark, [6]University Medical Center Groningen, Groningen, Netherlands, [7]Aarhus University Hospital, Urology, Aarhus, Denmark, [8]Aarhus University Hospital, Clinical medicine, Aarhus, Denmark

Background: The introduction of expanded criteria donors and donation after circulatory death have increased availability of kidneys, but also exposed a vital need for better organ quality measures. For now, there is no objective method available, resulting in possibly poor kidney transplants or potential grafts being discarded. To diminish organ waste and utilize available kidneys, we aim to develop a method to objectively quantify kidney quality through the combination of normothermic machine perfusion (NMP), nanoparticle-based biosensors, and near-infrared-fluorescence (NIRF) kidney imaging.

Ex-vivo normothermic machine perfusion creates a unique opportunity for kidney quality assessment prior to transplantation. Nanoparticles (NPs) are vehicles that can deliver molecules to cells via the active process of endocytosis. We are investigating two strategies to assess kidney cell vitality using NPs. 1) Administration of NPs loaded with a self-quenching NIRF dye, that will emit fluorescent light upon release in the cytoplasm, and 2) Administration of NPs loaded with mRNA encoding a NIRF-protein. Hypothesized that viable cells are more metabolically active, they will have superior nanoparticle uptake and mRNA translation, leading to a higher fluorescent signal. Thus, detected fluorescence could function as a non-invasive read out for kidney vitality.

Methods: Nephrectomy was performed on pigs to acquire human sized kidneys, which were preserved at static cold storage before transferred to NMP. In total 75 minutes of warm ischemia was used to induce kidney damage. NMP was performed with a red-blood-cell-based perfusate containing a mixture of nutrients, electrolytes, and multivitamins to meet metabolic requirements. NMP was performed up to five hours, at sinusoidal pressure of 100/60 mmHg using a centrifugal pump at 60 bpm. Real time whole kidney fluorescent imaging was performed using a laser and camera connected to the NMP setup.

Results/Conclusions: First data have proven nanoparticle uptake and detection of fluorescence by real-time whole kidney imaging. The fluorescent signal accumulated in time and was mainly present in the renal cortex. These results were achieved using both approaches, but the exact diagnostic value will be explored through studies investigating healthy vs damaged kidneys.

OP 03

EFFECTS OF FECAL MICROBIOTA TRANSPLANTATION ON CHRONIC KIDNEY DISEASE: AN INNOVATIVE APPROACH

Gianvito Caggiano[1], Mariavittoria D'acierno[2] [3], Consiglia Longobardi[3], Alessandra Stasi[1], Paola Pontrelli[1], Elena Squiccimarro[1], Antonio Miele[4], Ines Simeone[5], Michele Ceccarelli[4] [6], Giovambattista Capasso[2], Loreto Gesualdo[1], Anna Iervolino[2] [7]

[1]Nephrology, Dialysis and Transplantation Unit, Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy, [2]Carebios, Campus Regi Biologia s.r.l., Ariano Irpino, Italy, [3]University of Campania "Luigi Vanvitelli", Department of Mental, Physical Health and Preventive Medicine, Naples, Italy, [4]Biogem Institute of Molecular Biology and Genetics, Ariano Irpino, Italy, [5]Center for Genomic Science of IIT@SEMM - Istituto Italiano di Tecnologia, Milan, Italy, [6]University of Naples "Federico II", Department of Electrical Engineering and Information Technology

(DIETI), Naples, Italy, [7]*University of Campania "Luigi Vanvitelli", Department of Medical Translational Sciences, Naples, Italy*

Background: Recently, a large amount of data supports that the progression of chronic kidney disease (CKD) to end-stage renal disease (ESRD) is closely associated to the uremic toxins accumulation in blood and other compartments. This accumulation is strongly related to the production of toxic metabolites derived from gut microbiome accompanied by their inadequate clearance by injured kidneys. In this scenario, the need is felt to set up multifaceted approaches for dysbiosis management. Among several strategies, the fecal microbiota transplantation (FMT), currently used in the eradication of recurrent Clostridium difficile infections, could represent a promising novel therapy in the management of CKD-associated dysbiosis. In this study, we explored the potentialities of FMT in lowering the progression of kidney disease in mice with CKD.

Methods: In *in vivo* study, C57BL/6J mice were randomized into four groups: control, CKD, CKD +Antibiotics (AB) and CKD+AB+FMT. Renal failure in mice was induced by adenine oral gavage administration (50 mg/kg body weight) daily for 28 days. Consecutively, in order to reset the gut flora an antibiotic treatment was performed by using a cocktail of ampicillin + vancomycin + neomycin + metronidazole for a total of ten days prior to FMT treatment. Following antibiotic administration, the FMT from feces of human healthy donors was performed out every day for a total of seven days. At the end of experimental procedures, the structure of the intestinal microbiota, the physiological and metabolic profile were assessed in each group. Moreover, the kidney morphology was explored by the histological analysis.

Results: FMT improved the CKD-associated uremic toxicity in serum samples of uremic mice treated with FMT compared with CKD mice. Associated with the re-establishment of Bacteroidetes/ Firmicutes ratio, we observed a noticeable improvement of gut microbiota disturbance after FMT treatment. In spite of the kidney function was not recovered by the fecal transplantation, a decrease of interstitial fibrosis in renal environment was observed by the histological analysis.

Conclusions: These data indicate that the FMT could play a beneficial effect on the CKD progression. Further analyses are needed to better explore the FMT efficiency as a therapeutic option in CKD patients.

OP 04

INHIBITION OF THE ACTIVIN-SIGNALING PATHWAY DURING EX-VIVO NORMOTHERMIC MACHINE PERFUSION AS A NOVEL THERAPY IN KIDNEY TRANSPLANTATION

Thomas Weiss[1 2], Pernille Duedahl[1 2], Jesper Staulund[1 2], Henri Leuvenink[3], Andreas Lodberg[4], Camilla Merrild[1], Rikke Nørregaard[1], Anna Krarup Keller[1 5], Bente Jespersen[1 2], Marco Eijken[1 2]

[1]Aarhus University, Department of Clinical Medicine, Aarhus, Denmark, *[2]Aarhus University Hospital, Department of Renal Medicine, Aarhus, Denmark*, *[3]University Medical Center Groningen, Department of Organ Surgery and Transplantation, Groningen, Netherlands*, *[4]Aarhus University, Department of Biomedicine, Aarhus, Denmark*, *[5]Aarhus University Hospital, Department of Urology, Aarhus, Denmark*

Background: Ex-vivo normothermic machine perfusion (NMP) provides an opportunity for targeted ex-vivo pharmacological intervention before organ transplantation. Activin is a small protein secreted by a variety of tissues including the kidney. It has recently become evident that the activin-signaling pathway is a key player in the initiation of acute kidney injury and development of related renal fibrosis. Blocking activin signaling in the kidney could therefore be a promising therapeutic strategy in kidney transplantation. Systemic treatment with activin type II receptor blocking antibodies such as bimagrumab have shown to be safe in phase II clinical trials for the treatment of diabetes and muscle waste. The aim of this project is to evaluate the feasibility of a biological-based therapeutic intervention during kidney-NMP using activin type II receptor blocking monoclonal antibodies (αACTRII) with the intention to block activin signaling during NMP as well as in the first phase after transplantation.

Methods: In total, seven pairs of porcine kidneys were exposed to 75 minutes of warm ischemia followed by 16 hours of hypothermic machine perfusion and six hours of NMP using erythrocyte-based perfusate. At the start of NMP, the two kidneys from the same pig were randomized to control or treatment with 2 mg of αACTRII administered directly by infusion via

the renal artery. Throughout NMP, perfusate, tissue, and urine were continously sampled to study biomarkers, drug biodistribution and NMP-pharmacokinetics. Following NMP, precision-cut kidney slices were prepared and cultured for 48 hours to study the impact of pre-treatment during NMP by analysing gene expression of inflammatory, fibrosis, and activin signaling related genes.

Results-conclusion: Treatment with 2 mg αACTRII antibodies had no adverse effect on basic NMP characteristics such as renal flow, oxygen consumption, renal resistance, and creatinine clearance. Addition of activin or αACTRII to the kidney slices significantly regulated fibrosis related genes, however, no clear difference was observed between control and pre-treated kidneys. Currently, all other analyses regarding, biomarker levels, kidney gene expression, and αACTRII levels are ongoing.

OP 05

HUMAN TRANSPLANT KIDNEYS ON NORMOTHERMIC MACHINE PERFUSION DISPLAY ENDOCRINE ACTIVITY

Hui Lin[1], Zhaoyu Du[1], Dafsy Bouari[2], Elsaline Rijkse[2], Iacopo Cristoferi[2], Anja Obser[3], Jan Czogalla[3], A.H.Jan Danser[1], Robert Minnee[2], Martin Hoogduijn[1]

[1]Erasmus University Medical Center, Internal Medicine, Rotterdam, Netherlands, *[2]Erasmus University Medical Center, Surgery, Rotterdam, Netherlands*, *[3]University Medical Center Hamburg-Eppendorf, Medicine, Hamburg, Germany*

Background: Normothermic (37°C) machine perfusion (NMP) is an alternative to currently used hypothermic (4°C) machine perfusion (HMP) for donor kidney preservation before transplantation. Contrary to HMP, NMP allows for functional assessment of donor kidneys as the normothermic conditions allow for metabolic activity. The kidneys are key producers of hormones. Yet, it remains unknown whether donor kidneys during NMP display endocrine functions. We, therefore, investigated the release of prorenin/renin, erythropoietin (EPO), vitamin D, and urodilatin by kidneys on machine perfusion.

Methods: Ten donor kidneys were subjected to HMP followed by 2h of oxygenated NMP before transplantation. NMP perfusate was collected at three time points (0h, 1h, 2h) for the measurements of prorenin/renin, EPO, and vitamin D, and urine samples were collected for urodilatin measurement. Ten HMP perfusate samples were collected for the same measurements.

Results: Median release rates of prorenin (146 [Interquartile Range (IQR) 25-263] ng/hour) and renin (189 [IQR 90-277] ng/hour) in the first hour of NMP were 63- and 28-fold higher than that in HMP perfusates respectively (p=0.0029 and p<0.0001). Median renin release rate showed a 2.8-fold downregulation during the second hour of NMP compared to the first hour. EPO was secreted by kidneys on both HMP (9 [IQR 4-23] mIU/min) and NMP (1st hour: 14 [IQR 8-48] mIU/min; 2nd hour: 15 [IQR 6-92] mIU/min) without significant differences. Active vitamin D was undetectable in HMP perfusate samples, while the median secretion rates of vitamin D were 49 (IQR 29-79) and 29 (IQR 8-52) pmol/hour in the first and second hour of NMP respectively (p=0.0003 and p=0.0035). Seven donor kidneys produced urine during NMP and displayed detectable urodilatin levels. We then investigated whether there were correlations between the hormone releasing capacity and donor type. Interestingly, donation after brain death kidneys significantly released less EPO during the second hour of NMP (p=0.033) and more active vitamin D during the first and second hour of NMP (p=0.017 and p=0.017) than donation after circulatory death kidneys.

Conclusions: Human transplant kidneys release hormones during NMP. Hormone release may be used as a tool to assess kidney function ex vivo.

OP 06

NEUTROPHIL EXTRACELLULAR TRAPS REMOVAL DURING EX VIVO LUNG PERFUSION IMPROVES LUNG FUNCTION IN A PORCINE LUNG INJURY MODEL

Margareta Mittendorfer[1 2 3 4], Leif Pierre[1 3 4], Andrew Aswani[5 6], Gunilla Kjellberg[7], Franziska Olm[1 3 4], Sandra Lindstedt[1 2 3 4]

[1]Skåne University Hospital, Department of Cardiothoracic Surgery and Transplantation, Lund, Sweden, *[2]Lund University, Department of Clinical Sciences, Lund, Sweden*, *[3]Lund University, Lund Stem Cell Center, Lund, Sweden*, *[4]Lund University, Wallenberg Center for Molecular Medicine, Lund, Sweden*, *[5]Guy's and St Thomas' NHS Foundation Trust, Department of Critical Care, London, United Kingdom*, *[6]Santersus AG, Zurich, Switzerland*, *[7]Uppsala University Hospital, Department of*

Thoracic Surgery and Anaesthesiology, Uppsala, Sweden

Background: A major problem in lung transplantation that urgently needs to be addressed is the shortage of donor lungs. Many potential donor lungs are discarded due to gastric content aspiration induced acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). The reduction of Neutrophil Extracellular Traps (NETs) from the blood could be used as a therapeutic option due to their involvement in the inflammatory pathway of ALI. One such therapeutic option might be a NucleoCapture device which selectively removes NETs from the blood by utilizing human histone H1.3 protein conjugated to polymer beads. In this study, we investigated the effect of the removal of NETs during ex vivo lung perfusion (EVLP) on porcine aspiration damaged lungs.

Methods: 12 healthy pigs were stratified into two groups, treated (n=6) and not treated (n=6). All animals received 4ml/kg gastric content (pH=2) to induce ALI by distributing the gastric content equally between the different lung segments using a bronchoscope. Blood gas values, chest x-ray imaging and histological examination were used to confirm the development of mild to moderate ARDS over the course of 6 hours. Lungs were subsequently explanted *en bloc* and connected to an EVLP circuit for 4 hours. Treated lungs were placed in line with a NucleoCapture device (Santersus AG) connected to the EVLP circuit and the non-treated group underwent the same EVLP protocol without the device.

Results: ARDS was induced in all animals as confirmed by infiltration on chest x-ray, histopathological examination and by PaO2/FiO2 ratio. Following treatment with the NucleoCapture device during 4 hours of EVLP, the PaO2/FiO2 ratio was significantly increased compared to non-treated lungs and was found to surpass the threshold values suitable for transplantation. Further, treated lungs were macroscopically improved compared to both initial injured lungs as well as non-treated lungs.

Conclusions: Treatment of aspiration injured lungs with a NucleoCapture device to remove NETs from the EVLP circuit resulted in macroscopic and oxygenation improvement of otherwise damaged donor lungs. The results may show promise for increasing the lung donor pool.

OP 07

DECELLULARIZED HUMAN VEINS AS MODEL FOR VASCULAR REPAIR AND ENDOTHELIAL CELL CHIMERISM IN ORGANTRANSPLANTATION

Hector Tejeda Mora[1], Yvette den Hartog[1], Ivo Schurink[2], Monique Verstegen[2], Jeroen de Jonge[2], Robert Minnee[2], Martijn van den Hoogen[2], Carla Baan[1], Martin Hoogduijn[1], Luc van der Laan[2], Jorke Willemse[2]

[1]Erasmus MC Transplant Institute, Dept. of Internal Medicine, Rotterdam, Netherlands, *[2]Erasmus MC Transplant Institute, Dept. of Surgery, Rotterdam, Netherlands*

Background: In transplantation, the endothelial lining is the first barrier between the donor organ and recipient immune system. Damaged endothelium aggravates inflammation and causes graft rejection. Restoration of the endothelial barrier function is thus crucial for the normal graft performance after transplantation. Here we prove that re-endothelialization of acellular blood vessels using venous-derived endothelial cells (EC) can repair the vascular barrier function and restore innate immune function of the endothelium.

Methods: Human veins (n=19) from deceased donors were decellularized by submersion in Triton X-100, ammonia and DNase. Efficacy of the process was evaluated by DNA removal, but preservation of ECM collagen (type I and IV). Decellularized veins were subsequently repopulated with EC. The reconstructed EC monolayer was analysed using confocal microscopy, trans-endothelial electrical resistance (TEER), and dextran permeability. EC functionality was evaluated via nitric oxide production. The innate immune function was assessed by co-culture with THP-1 monocytic cells. EC repair was evaluated after targeting HLA specific EC by complement activation in an alloimmune response manner.

Results: Veins were fully decellularized, demonstrated by the removal of cellular components, and dsDNA (before: 83.8±29.0 , after:13.0±6.5 ng/mg). Histological integrity was preserved, as well as collagens. Confocal microscopy showed the formation of a confluent cell monolayer as soon as 24h after seeding. After 28 days of culture, repopulated scaffolds remained confluent. The constructs had TEER measurements above background of 15.1±12.2 Ω·cm²; reduced dextran permeability compared to decellularized veins (4.7-fold); and showed nitric oxide production. The innate immune

barrier function was demonstrated by THP-1 cell adhesion, transmigration and polarization towards macrophage phenotype. Finally, chimeric EC showed repair capacity after re-occupy the exposed ECM caused by complement activation.

Conclusions: These results proved the feasibility to repair in the context of humoral anti-donor responses in vitro. This highlights the potential of autologous EC for vascular repair, reducing the immunological burden of allogeneic grafts.

OP 08

DNA VECTOR-INDUCED EPO-OVEREXPRESSING KIDNEY ORGANOIDS INCREASE HEMATOCRIT LEVELS

Zhaoyu Du[1], Amanda Bas-Cristóbal Menéndez[1 2], Manuela Urban[3], Thierry van den Bosch[4], Marian Clahsen-van Groningen[4], Joost Gribnau[5], Marlies Reinders[1], Carla Baan[1], Richard Harbottle[3], Martin Hoogduijn[1]

[1]Erasmus MC Transplant Institute, University Medical Center, Department of Internal Medicine, Rotterdam, Netherlands, *[2]Sophia Children's Hospital, Erasmus University Medical Center, Department of Pediatrics, rotterdam, Netherlands*, *[3]German Cancer Research Center (DKFZ), DNA Vectors Lab, Heidelberg, Germany*, *[4]Erasmus MC, University Medical Center, Department of Pathology, rotterdam, Netherlands*, *[5]Department of Developmental Biology and iPS Core Facility, Erasmus MC, University Medical Center, Rotterdam, Netherlands*

Background: Human induced pluripotent stem cell (iPSC) derived kidney organoids are novel research tools for studying kidney development, disease and physiology. In addition to its filtration function, the kidney also has an important endocrine role, including secretion of erythropoietin (EPO) under hypoxia. Our aims were to generate kidney organoids with a functional EPO endocrine system, to study effects of EPO on kidney organoid phenotype and examine the physiological effects of EPO-producing organoids.

Methods: By using a scaffold matrix attachment region (S/MAR) DNA vector, persistent expression of EPO in iPSC was achieved. EPO-overexpressing (EPO-oe) organoids were characterized using markers including PODXL (podocyte), Villin-1 (proximal tubule), E-cadherin (distal tubule). To determine whether EPO released by kidney organoids has physiological effects, EPO-oe kidney organoids were subcutaneously implanted in immunodeficient mice and mouse hematocrit (HCT) levels were measured after 1 month.

Results: In control organoids, EPO was undetectable at both transcript and secreted protein level. After 24 hours-hypoxia, EPO were detected at a median 0.43 (interquartile range [IQR] 0.34-0.59) mIU/ml in supernatant. This low level of EPO is insufficient for functional studies. EPO-oe kidney organoids were generated and maintained high EPO mRNA expression for over 25 days. EPO levels in supernatants were 933 mIU/ day per EPO-oe kidney organoid. Immunohistochemistry (IHC) staining showed that EPO overexpressing kidney organoids formed similar kidney structures compared to control organoids and the expression of kidney specific markers PODXL, Villin-1 and E-cadherin was not changed. One month after implantation of organoids in mice, HCT levels were significantly increased in mice received EPO-oe organoids (48.0%, IQR 43.6-55.1%) compared to mice that received control organoids (40.10%, IQR 39.7-41.2%) (p=0.0092, n=9). Meanwhile, implanted EPO-oe organoids developed endothelial cells with elongated morphology and CD31 expression was 6.5-fold higher than in control organoids.

Conclusions: EPO-oe human kidney organoids show stable production of functional EPO. The implantation of EPO-oe organoids shows the potential of using human iPSC-derived kidney organoids for gene therapy.

OP 09

A BIOENGINEERED VASCULARIZED SPLEEN MATRIX AS AN ENDOCRINE NEO-PANCREAS

Louis Maistriaux[1 2], Matias Ramirez[2], Lies Fievé[1], Chae Hee-Young[3], Eliano Bonaccorsi Riani[2], Catherine Behets-Wydemans[1], Pierre Gianello[2], Benoit Lengelé[1]

[1]UCLouvain, IREC - Pole of Morphology, Brussels, Belgium, *[2]UCLouvain, Pole of Experimental Surgery and transplantation, Brussels, Belgium*, *[3]UCLouvain, IREC - Pole of Endocrinology, Brussels, Belgium*

Background: Diabetes is mainly treated with insulin injections, but pancreas or islets transplantations remain the best therapeutic options. However, they are clinically underused due to the side effects of immunosuppressive

drugs and pancreas donor shortage. To overcome this, a tissue engineering (TE) pathway was explored to regenerate a functional bioengineered endocrine graft using an acellular spleen matrix (DSM), easier to harvest and more available than pancreatic graft.

Methods: 44 vascularized rat spleen grafts were decellularized by detergents perfusion. Cell clearance and extracellular matrix (ECM) preservation were studied by histology, as well as quantification of DNA, ECM proteins, growth factors (GF) and residual SDS. Cytocomptability was evaluated with PrestoBlue Assay and Live/Dead after 7 days of culture of NIH-3T3 cells on DSM patches and 8 days of MIN-6 cells cultured in DSM-conditioned medium. Biocompatibility was analyzed after subcutaneous implantation of DSM or native tissue at 14 & 30 days; CD68 & CD31 cells infiltration were assessed by immunohistochemistry. Finally, MIN-6 cells were injected through the parenchyma or the splenic artery of whole DSM and cultured 5 days in perfusion bioreactor. Cell function was studied with Glucose-Stimulated Insulin Secretion test (GSIS).

Results: DSM showed a preserved 3D morphology and appeared white. DNA amount <50ng/mg and absence of cells in histology confirmed the cell clearance. Histology and immunostaining for collagens I & IV, fibronectin, laminin and ECM proteins assays confirmed the preservation of microarchitecture and ECM components but a decrease in GF. Non-toxicity of DSM was emphasized by low amount of SDS residues and similar viability of NIH-3T3 & MIN-6 cells. Biocompatibility was confirmed by an *in vivo* revascularization and a lower CD68 cells infiltration in DSM than native tissue. Both seeding methods allowed MIN-6 cells engraftment and formation of cell clusters into DSM cultured in bioreactor while keeping their insulin release during a perfused GSIS at day 5.

Conclusions: TE allowed to create vascularized DSM with preserved tissular architecture and ECM components while being biocompatible. Furthermore, DSM allows pancreatic cells growth and could be a future solution for pancreatic regeneration and transplantation.

MODERATED E-POSTERS

POS 01

CORONARY ALLOGRAFT VASCULOPATHY IS ASSOCIATED WITH DECREASED CD34+ PERIPHERAL CELL COUNT IN PATIENTS AFTER HEART TRANSPLANTATION

Gregor Poglajen[1] [2], Laura Poljancic[3], Rok Zbačnik[3], Sabina Frljak[1], Neža Žorž[1], Andraž Cerar[1], Gregor Zemljič[1], Renata Okrajšek[1], Miran Šebeštjen[1], Bojan Vrtovec[1]

[1]University Medical Center Ljubljana, Advanced Heart Failure and Transplantation Center, Ljubljana, Slovenia, [2]Faculty of Medicine, Department of Internal Medicine, Ljubljana, Slovenia, [3]University Medical Center Ljubljana, Institute of Radiology, Ljubljana, Slovenia

Background: Mechanisms of coronary allograft vasculopathy (CAV) after heart transplantation remain poorly understood. As $CD34^+$ cells represent one of the key determinants of coronary vascular homeostasis we investigated a potential association between CAV and $CD34^+$ cell count in heart transplant recipients.

Methods: In a single-center prospective cohort study we included 59 adult heart transplant recipients without history of congenital heart disease, multi-organ transplantation or oncologic therapy. All patients underwent coronary CT angiography and the presence of CAV was defined in accordance with the ISHT criteria. At the time of CT angiography, we collected blood samples and measured $CD34^+$ cell count using Beckman-Coulter Navios EX flow cytometry with standard antibodies according to ISAGE protocol as well as biomarkers of angiogenesis using Luminex assay kit.

Results: CAV was present in 15 patients (25%; Group A) and absent in 42 patients (75%; Group B). The two groups did not differ in age (62±11 years in Group A vs. 60±11 years in Group B, P=0.56), gender (male: 100% vs. 80% in Group B, P=0.07), heart failure etiology (ischemic: 53% vs. 43%, P=0.50), presence of hypertension (60% vs. 63%, P=0.80), diabetes (33% vs. 25%, P=0.54) or renal insufficiency (53% vs. 43%, P=0.50). Also, donor age (44±14 years in Group A vs. 43±12 years in Group B, P=0.66), allograft ischemic time (202±72 min vs. 190±68 min, P=0.57), tacrolimus trough levels (8.8±3.8 µg/L vs. 7.4±1.9 µg/L, P=0.14), MMF dose (2067±442 mg vs. 2178±606 mg; P=0.52) and statin therapy (86% vs. 75%; P=0.36) were comparable. While total leukocyte count was similar in both groups ($7.7\pm2.1 \times 10^9$/L in Group A vs. $6.8\pm2.2 \times 10^9$/L in Group B, P=0.60), we found significantly lower $CD34^+$ cell count in Group A compared to Group B ($1.33\pm0.45 \times 10^6$/L vs. $2.23\pm1.45 \times 10^6$/L, P=0.02). Conversely, VEGF serum levels were significantly lower in Group A than in Group B (0.09±0.06 ng/L vs. 0.14±0.09 ng/L; P=0.03).

Conclusions: Decreased $CD34^+$ cell count and increased VEGF serum levels appear to be associated with CAV in heart transplant recipients. Further studies are needed to investigate the potential of $CD34^+$ cells in prevention and treatment of CAV in this patient cohort.

POS 02

EARLY PHASE CLINICAL TRIALS USING BIO-ARTIFICIAL ORGAN TECHNOLOGY: A SYSTEMATIC REVIEW OF ETHICAL ISSUES

Dide de Jongh[1], Emma Massey[1], Antonia Cronin[2], Eline Bunnik[1]

[1]Erasmus MC, Rotterdam, Netherlands, [2]kings college hospital, London, United Kingdom

Background: Regenerative medicine has emerged as a potential response to the persistent problem of shortage of donor organs in the field of organ transplantation. Around the world, in preclinical research settings, bioartificial organs are being developed that can be used for transplantation into human recipients. Within a couple of years, first-in-humans and early-phase clinical trials are expected to be launched to test the safety and efficacy of these products in patients. In early-phase bio-artificial organ transplantation trials, research participants will be exposed to serious - known and unknown - risks. As of yet, there is no ethical guidance for the safe and responsible design and conduct of early-phase clinical trials of bioartificial organs. Therefore, when setting up or evaluating trials, research groups and research ethics review committees must look to adjacent fields of research, including regenerative medicine, tissue engineering, 3D bioprinting, organ transplantation, and cell-based therapy, for guidance.

Methods: We did a systematic literature review of adjacent fields of research, in which we identified and examined relevant ethical points to consider for early-phase clinical trials of transplantable bioartificial organs.

Results: 92 scientific peer-reviewed articles were included. Six themes were identified: cell source, risk-benefit assessment, patient selection, trial design, informed consent, and ethics oversight.

Conclusions: Overall, this systematic review reveals that further empirical research is needed, notably on patient perspectives, as well as ethical analysis, to help ensure the responsible development and conduct of clinical trials of bioartificial organs.

POS 03

THE ROLE OF THE COMPLEMENT SYSTEM IN LIVER ISCHAEMIA REPERFUSION INJURY

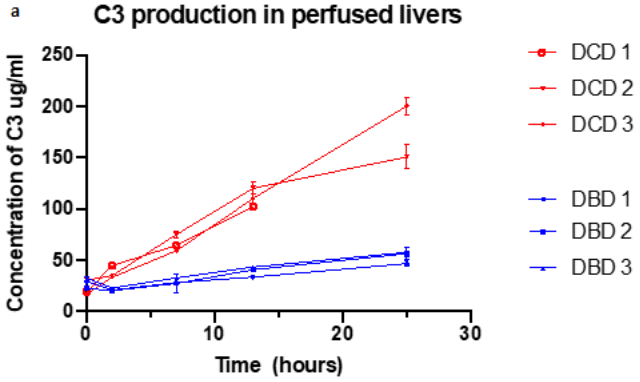
Balaji Mahendran[1] [2], Chloe Connelly[1], Samuel Tingle[1] [2], Lucy Bates[1], Beth Gibson[1], Emily Thompson[1] [2], Rodrigo Figueiredo[1] [2], James Clark[1], Olivier Govaere[1], Kevin Marchbank[1], Neil Sheerin[1] [3], Colin Wilson[1] [2]

[1]Newcastle University, Institure of Cellular Medicine, Newcastle, United Kingdom, [2]Newcastle upon Tyne Hospitals NHS Trust, HPB & Transplant Surgery, Freeman Hospital, Newcastle, United Kingdom, [3]Newcastle upon Tyne Hospitals NHS Trust, Department of Nephrology, Newcastle, United Kingdom

Background: The discrepancy between donor organs and recipients on the transplant waiting list has led to the use of livers from donors after circulatory death (DCD) organs. DCD liver grafts are at high risk of ischaemic cholangiopathy- similarly to ABO blood group mismatched liver transplants. We hypothesised that the complement system plays a key role in the pathogenesis of this disease.

Methods: Livers declined for clinical transplantation were perfused for up to 12 hours with anti-coagulated packed red cells at 37° using a modified cardiopulmonary bypass circuit. The perfusion pressures were fixed (mean hepatic artery pressure 75mmHg, portal vein 4-5mmHg) to maintain physiological perfusion. Levels of circulating complement components, tissue binding of complement proteins, and organ damage markers were measured in the perfusate and on tissue samples.

Results: 6 livers (3 DBD, 3 DCD) were perfused, with no significant differences in ischaemia times. There was a significant difference in the circulating levels of C3 between DBD and DCD livers ($p<0.05$) at 12 hours (Figure 1).



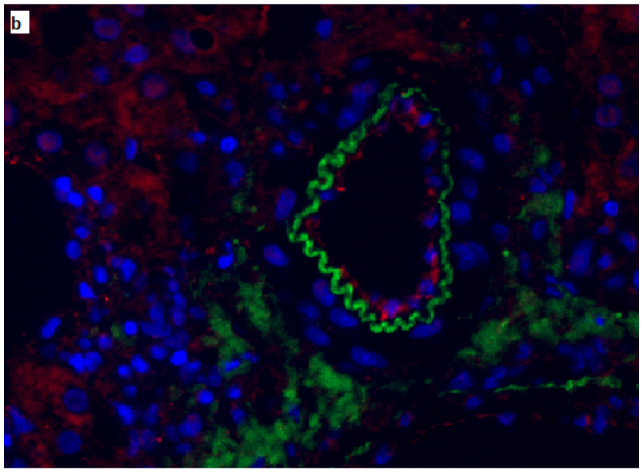


Figure 1a - Graph of C3 production in machine perfused livers
Figure 1b - Co-localised immunofluoresence image of a machine perfused liver section taken at 10 hours post perfusion. Green - C5b-9, Red - PECAM portal arterial endothelial marker, Blue - DAPI nuclear staining

Whilst there was no difference in the levels of circulating C5b-9 (4457 ng/ml vs 3233 ng/ml, $p=0.66$) there was an increase in deposition of C5b-9 on the portal arterial endothelium in DCD livers (1.245 R.U vs 1.442 R.U, $p=0.0341$).

Conclusions: These results show that the complement system may play an important role in the initial stages of ischaemia-reperfusion injury within the liver. The complex interplay between regulatory and effector proteins within the complement system necessitates further study to explain the discordance seen between the circulating levels of C3 and C5b-9.

POS 04

PREDICTION OF VESSEL OCCLUSION DURING RECELLULARIZATION USING A COMPUTATIONAL FLUID DYNAMICS MODEL

Van Lap Nguyen[1], Hiromichi Obara[1]

[1]Tokyo Metropolitan University, Department of mechanical systems design, Tokyo, Japan

Background: The generation of the functional human-scaled organ by decellularization and recellularization processes is a promising approach for addressing the organ shortage. Accordingly, the donor cells are removed from human or non–human organs, then the patient's cells are seeded into the decellularized organ. The function of the generated organ is related to the number and the distribution of seeded cells. However, because of the occlusion of vessels, it is not straightforward to achieve a uniform distribution of cells. In this study, we aimed to quantify the occurrence of vessel occlusion during recellularization under different seeding conditions.

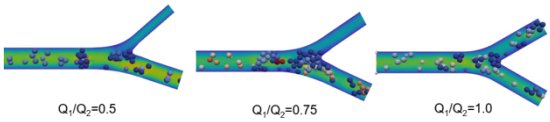
Methods: A combined model that coupled computational fluid dynamics (CFD) and discrete element method (DEM) was employed to describe the vessel occlusion phenomenon. Cells were, presented as discrete phase, solved by DEM solver; and the culture medium was, modelled as fluid phase, solved by CFD solver.

Results: The model indicated that the occurrence of vessel occlusion increased as cell concentration increased, especially a rapid occlusion occurred as the cell concentration was above 4%. In addition, the asymmetric bifurcating vessel with the ratio of outlet flow rate (Q1/Q2) resulted in a high occurrence of occlusion (Figure.1) as gravity was not considered, this is because of the high chance of interaction between cell and vessel wall at the bifurcation apex. As the ratio of Q1/Q2 = 0.5, cells tended to concentrate in the higher flow rate daughter branch, leading to the parenchyma in the side of the lower flow rate branch might be not repopulated by cells. This explains the non – uniform distribution along with vessel occlusion.

Conclusions: In this study, the dynamic of vessel occlusion during the recellularization process was revealed. The results show that the occurrence of vessel occlusion depends on cell concentration, the branching shape of the vessel, and the direction of gravity. The predictions can help to select the optimum parameters for the recellularization process.

Keywords: Recellularization and vessel occlusion

Figure. 1. The distribution of cell at 6% of cell concentration in the vessel with different branching ratio



POS 05

ORGAN VIABILITY ASSESSMENT WITH IMAGING MEASUREMENT TECHNOLOGY DURING MACHINE PERFUSION FOR PORCINE LIVER DONATED AFTER CARDIAC DEATH

Hiromichi Obara[1], Yuga Okazawa[1]

[1]Tokyo Metropolitan University, Hachioji, Japan

Background: A machine perfusion is a promising strategy to preserve organs donated after cardiac death (DCD). It provides some opportunities to preserve, improve and assess the graft viability prior to transplantation. The assessment methods for graft viability during machine perfusion are important to extend the donor criteria and the total number for transplantation. In this study, we suggest novel imaging measurement technology with thermal and infrared cameras to investigate distribution of the ischemic injury part to predict organ qualities.

Methods: Pigs were employed to harvest a liver graft under several warm ischemic time conditions(0,45,60.) The system for machine perfusion was consisted of a highspeed thermal camera to measure temperature distributions of the organ and a high sense camera for near infrared to detect ICG florescent dynamics. We perfused porcine livers injected ICG through the portal vein during perfusion under hypothermic, subnormothermic and normothermic conditions. The fluorescence image measurements were analysed to evaluate the flow and metabolism in the organs.

Results: As an example, results from ICG are described here. In the images taken 180 sec after the addition of ICG, different fluorescence dynamics were observed depending on the individual. In particular, the fluorescence area and the intensity of fluorescence differ greatly between the experimental conditions, indicating the differences in the flow conditions. After 300 sec, the fluorescence spread in all livers, and the concentration diffusion of fluorescence

intensity spread from capillaries to the tissues was observed.

Conclusions: The potential of imaging measurement technology was presented for improvement of the machine perfusion and regenerative medicine.

Keywords: Machine Perfusion, Viability assessment, organ preservation

POS 06

EFFECT OF PERFUSATE COMPOSITION DURING LONG-TERM PERFUSION OF LIVERS USING A PRECISION-CUT LIVER SLICE MODEL

Julia Hofmann[1], Andras T. Meszaros[1], Verena Hackl[1], Nadine Ortner[2], Christina Bogensperger[1], Theresa Hautz[1], Stefan Schneeberger[1], Thomas Resch[1]

[1]Medical University Innsbruck, Innsbruck, Austria, *[2]University of Innsbruck, Innsbruck, Austria*

Background: Long-term normothermic machine (NMP) perfusion may open the opportunity to regenerate marginal organs *ex vivo* and thus help to overcome organ shortage. However, electrolytes in the perfusate are increasing during isolated perfusion since filtration is lacking. Thus, we developed a precision-cut liver slice (PCLS) model to evaluate the effect of changing perfusate composition.

Methods: 8 mm punch biopsies were obtained of cold preserved porcine livers prior they underwent normothermic machine perfusion. PCLS (300 μM) were generated using a vibratome (Leica 1200S) and were cultivated for 7 days in 24-well plates on a rocking platform. Different conditioned DMEM media containing defined concentrations of Na^+ (160 or 165 mmol/L), K^+ (7 or 15 mmol/L) and urea (200 or 400 mg/dL) were utilized. Tissue viability and functionality were assessed by MTS assay and albumin production, cell damage was assessed by LDH secretion into the cell culture media.

Results: Viable and metabolically active PCLS could be generated and cultivated for 7 days irrespective of the utilized media. Viability was maintained for all conditions with no significant differences compared to the control group and compared to viability after slicing. Albumin production was observed for all conditions with no significant differences, confirming the results of the MTS assay. However, due to the slicing process LDH release into the supernatant was observed. In the course of cultivation, LDH levels remained stable for all conditions. No significant differences between conditioned media and control were found, indicating elevated electrolytes or urea cause no further damage to liver tissue.

Conclusions: Na^+, K^+ and urea levels above the physiological range do not cause damage to PCLS. Thus, elevated perfusate levels of those analytes may not lead to tissue injury during long-term machine perfusion.

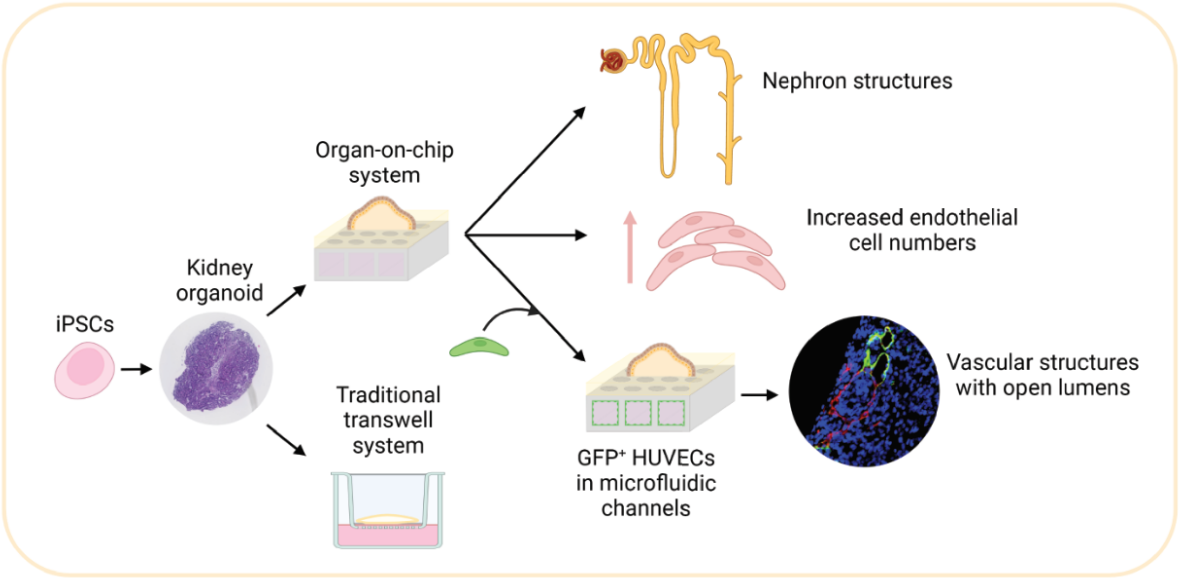
POS 07

CREATING A KIDNEY ORGANOID-VASCULATURE INTERACTION MODEL USING A NOVEL ORGAN-ON-CHIP SYSTEM.

Amanda Bas-Cristóbal Menéndez[1], Zhaoyu Du[2], Thierry van den Bosch[3], Hui Lin[2], Jaap Mulder[4], Martin Hoogduijn[2]

[1]Erasmus MC, Internal medicine / pediatrics, Rotterdam, Netherlands, *[2]Erasmus MC, Internal medicine, Rotterdam, Netherlands*, *[3]Erasmus MC, Pathology, Rotterdam, Netherlands*, *[4]Erasmus MC, Pediatrics, Rotterdam, Netherlands*

Graphical abstract:



Background: Kidney organoids derived from human induced pluripotent stem cells (iPSCs) have proven to be a valuable tool to study kidney development and disease. However, the lack of vascularization in kidney organoids often leads to insufficient oxygen and nutrient supply and therefore prevents them from reaching sequential stages of maturation. Vascularization of the organoids has previously been achieved by implantation into animal models, however, the vasculature in these models arises mostly from animal host tissue. Our aim is to transition from an *in vivo* system towards an *in vitro* model that fulfils the advantages of vascularization whilst being fully human-cell-derived by using a silicon-based organ-on-chip system.

Methods: Our organ-on-chip consists of a microfluidic chip with three perfusable microchannels that connect to a culturing chamber through a porous membrane. Human umbilical vein endothelial cells (HUVECs) were seeded in the channels to create synthetic 3D vessels.

Results: This model was proven to support culturing of kidney organoids, which presented glomerular structures, proximal tubuli and distal tubuli. We also showed that organoids cultured on chip presented larger endothelial populations as well as improved vascular endothelial tissue maturation in comparison to traditional protocols. A colocalization analysis of endothelial markers CD146 and CD31 demonstrated that vascular tissue in kidney organoids cultured on chip presented marker expression that correlates with intermediate endothelial maturation. Moreover, we observed migration and proliferation of the HUVECs cultured in the perfusable channels of the chip inside the organoid tissue, where they interconnected with endogenous endothelial cells and formed vessel-like structures presenting open lumens.

Conclusions: Our model presents the first successful vascularization of kidney organoids in co-culture with HUVECs in an organ-on-chip system. Moreover, we proved flow also improved vascular tissue maturation patterns in kidney organoids. Although further research is necessary to generate organoids in this system from earlier stages, we believe this model constitutes a valuable tool for the study of kidney-vasculature interaction with applications such as pre-clinical drug testing.

POS 08

MODELLING KIDNEY FIBROSIS IN HUMAN KIDNEY ORGANOIDS

Shengbing LI[1], Thierry van den Bosch[2], Zhaoyu Du[1], Amanda Bas-Cristóbal Menéndez[1], Hui Lin[1], Carla Baan[1], Marlies Reinders[3], Martin Hoogduijn[1]

[1]transplant institute, Rotterdam, Netherlands, [2]Department of Pathology, Rotterdam, Netherlands, [3]internal medicine, Rotterdam, Netherlands

Background: Kidney transplantation is the treatment of choice for end-stage kidney disease. However, ischemia during transplantation, allo-immune responses, and ageing after transplantation drive transplant kidney fibrosis. The lack of experimental models for human kidney fibrosis limits the development of anti-fibrosis medicines. Kidney

organoids may represent a tool for studying fibrosis. The aim of this study was to set up a kidney organoid fibrosis model. Hence, we treated kidney organoids with hypoxia, interleukin-1β (IL-1β) or/and prolonged culture to mimic ischemia, inflammation and aging and explored the degree of fibrosis induced by these methods.

Methods: Human iPSC-derived kidney organoids were stimulated with 1% O_2 48h, 100ng/ml IL-1β 96h or the combined stimulations at day 12 or day 14 of differentiation. Subsequently, organoids were cultured under standard conditions for 7 days or 14 days. Organoids were analyzed through RT-PCR for fibrosis markers and kidney structural markers were stained through immunohistochemistry.

Results: Fibronectin 1(FN1), tenascin C(TNC) and transforming growth factor beta (TGF-β) mRNA was significantly upregulated upon exposure of organoids to combined hypoxia and IL-1β. Collagen type 1A1(COL1A1) was upregulated significantly upon 14 days prolonged culture even in absence of hypoxic or inflammatory stimulation. Immunohistochemistry demonstrated kidney structures including WT1+ glomeruli, Villin-1+ proximal tubules and ECAD+ distal tubules in all conditions, but after 14 days prolonged culture, these structures were partly replaced by COL1A1+ cells.

Conclusions: Our results show that organoids display fibrosis after combined hypoxia and IL-1β stimulation and 14 days prolonged culture with or without stimulation. These models can be used for studying fibrosis processes and anti-fibrosis treatment in human kidney.

POS 09

Withdrawn

POS 10

THE LNCRNA HOTAIR CONTROLS THE SELF-RENEWAL, CELL SENESCENCE, AND SECRETION OFANTIAGING PROTEIN A-KLOTHO IN HUMAN ADULT RENAL PROGENITOR CELLS

Angela Picerno[1], Francesca Giannuzzi[1], Claudia Curci[1], Giuseppe De Palma[2], Maria Giovanna Dl Chiano[3], Simona Simone[4], Rossana Franzin[3], Anna Gallone[3], Vito Francesco Dl Lorenzo[2], Alessandra Stasi[3], Giovanni Battista Pertosa[3], Carlo Sabbà[1], Loreto Gesualdo[3], Fabio Sallustio[1]

[1]University of Bari Aldo Moro, Department of Interdisciplinary Medicine, Bari, Italy, *[2]IRCCS Istituto Tumori "Giovanni Paolo II, Bari, Italy*, *[3]University of Bari Aldo Moro, Bari, Italy*, *[4]Azienda Ospedaliero Universitaria Consorziale Policlinico, Bari, Italy*

Background: The long non-coding RNAs (lncRNA) play an important role in several biological processes including some renal diseases. Nevertheless, little is known on lncRNA that are expressed in healthy kidney and involved in renal cell homeostasis and development, and even less is known about lncRNA involved in the maintenance of human adult renal stem/progenitor cells (ARPCs) that have been shown to be very important for renal homeostasis and repair processes.

Methods: Whole-genome lncRNA expression was performed by Agilent microarray. lncRNA expression was validated by Real-time PCR. CRISPR/Cas9 system has been used to knock-down HOTAIR lncRNA. SA-β-Gal experiments were used to evaluate cellular senescence in normal ARPCs and ARPCs knock-out for HOTAIR. By ELISA, it was evaluated the expression of secreted anti-aging protein Klotho. FACS was applied to measure CD133 and protein p15 expression in normal and transfected cells. Chromatin immunoprecipitation assay (chIP) was used to evaluate H3K27me3 in the promoter of p15.

Results: We studied the lncRNA profile of renal proximal tubular cells (RPTEC) and of tubular ARPCs. We found 611 lncRNAs specifically expressed in ARPCs compared to RPTECs (FC> 2; FDR<0.05). Among the most significantly modulated lncRNAs, HOX Transcript Antisense RNA (HOTAIR) was highly expressed in ARPCs (FC = 15; $p < 0.001$). The silenced lines for HOTAIR immediately assumed a senescent phenotype confirmed by the beta-galactosidase assay and decreased proliferation (60% decrease, $p<0.001$). Moreover, we found that the constitutional, functional, and inverse-senescence marker CD133+ was downregulated in knock-out cells (Fold change = 15; $p < 0.01$) and that ARPCs expressed high levels of the α-Klotho anti-aging protein, regulated by HOTAIR, 2.6-fold higher compared to RPTECs. Finally, we showed that HOTAIR exerts its function through the epigenetic silencing of the cell cycle inhibitor p15 inducing the trimethylation of the histone H3K27.

Conclusions: These data demonstrated that HOTAIR regulates the self-renewal capacity of ARPCs and and prevents them from becoming senescent in the short term. Moreover, HOTAIR influences ARPC ability to secrete high levels of α-Klotho, influencing its levels in surrounding tissues modulating, consequently, the kidney aging.

POS 11

INTRAPORTAL HUMAN ALLOGENEIC MESENCHYMAL STEM CELLS INFUSION FOR POSTHEPATECTOMY LIVER FAILURE PREVENTION: THE PILOT CASE SERIES EXPERIENCE.

Denis Efimov[1], Aliaksei Shcherba[1], Sergey Korotkov[1], Evgeniya Primakova[1], Ala Symanovich[1], Paul Bychkovskiy[1], Ekaterina Nazarova[1], Svetlana Krivenko[1], Oleg Rummo[1]

[1]Minsk scientific and practical center for surgery, transplantology and hematology, Misnk, Belarus

Background: Mesenchymal stem cells (MSCs) have been demonstrated to stave off the progression of liver injury and potentially improve liver function

The aim of this study was to assess feasibility, safety and potential efficacy of the allogeneic adipose tissue MSCs local intraportal infusion for PHLF prevention

Methods: We report an experience of 4 prospective clinical cases of intraportal MSCs infusion during major hepatectomy. Inclusion criteria: major hepatectomy (more than 4 segments), future liver remnant volume <30%, benign indication for liver resection in adults. Exclusion criteria: partial portal vein thrombosis (PVT), malignant tumor, portal hypertension (> 20 mmHg). Allogeneic human MSCs in dose of 20 million cells were infused intraportally during major hepatectomy after direct pressure measurement subsequent to surgical removal of specimen before wound closure. Serological probes from peripheral vein before, on the 1st, 4th and 7th postoperative days (POD) as well as inreoperative probes from portal and hepatic veins have been taken for TGF-b, EGF, HGF, IL-6, TNF-a, M30, M65 evaluation. Liver remnant biopsy was taken before wound closure. Ultrasound was performed postoperatively to exclude PVT. PHLF was assessed under ISGLS criteria.

Results: 3 out of 4 patients had got Alveococcosis of the liver, and one – multiple focal nodular hyperplasia. There were 1 ALPPS procedure and 1 portal vein embolization before resection. During ALPPS procedure MSCs were infused twice during the first and the second stage of liver resection. There were no PHLF grade C (ISGLS), 1 patient deceased due to PDR Klebsiella cholangitis and sepsis on the 15th POD. No PVT or other mscs infusion complications were revealed.

Conclusions: Clinical intraportal MSCs infusion during major hepatectomy is safe intervention with no severe adverse events revealed. The future clinical investigation is needed to provide evidence of PHLF prevention by intraportal MSCs infusion.

POS 12

HIGH THROUGHPUT PRODUCTION OF EXTRACELLULAR VESICLES (EVS) FROM IMMORTALIZED WHARTON'S JELLY MESENCHYMAL STROMAL CELLS (IWJ-MSC) (EVS)

Sergio Garcia Garcia[1] [2], Marta Sanroque-Muñoz[1], Marta Clos-Sansalvador[1] [2], Marta Monguió-Tortajada[3] [4], Miriam Moron Font[1], Francesc E. Borras[1], Marcella Franquesa[1]

[1]REMAR-IGTP Group, Germans Trias i Pujol Research Institute (IGTP) & Nephrology Department, University Hospital Germans Trias i Pujol (HUGTiP), Badalona, Spain, [2]Autonomous University of Barcelona, Department of Cell Biology, Physiology and Immunology, Bellaterra, Spain, [3]ICREC Research Program, Health Science Research Institute Germans Trias i Pujol (IGTP), Badalona, Spain, [4]Cardiology Service, University Hospital Germans Trias i Pujol (HUGTiP), Badalona, Spain

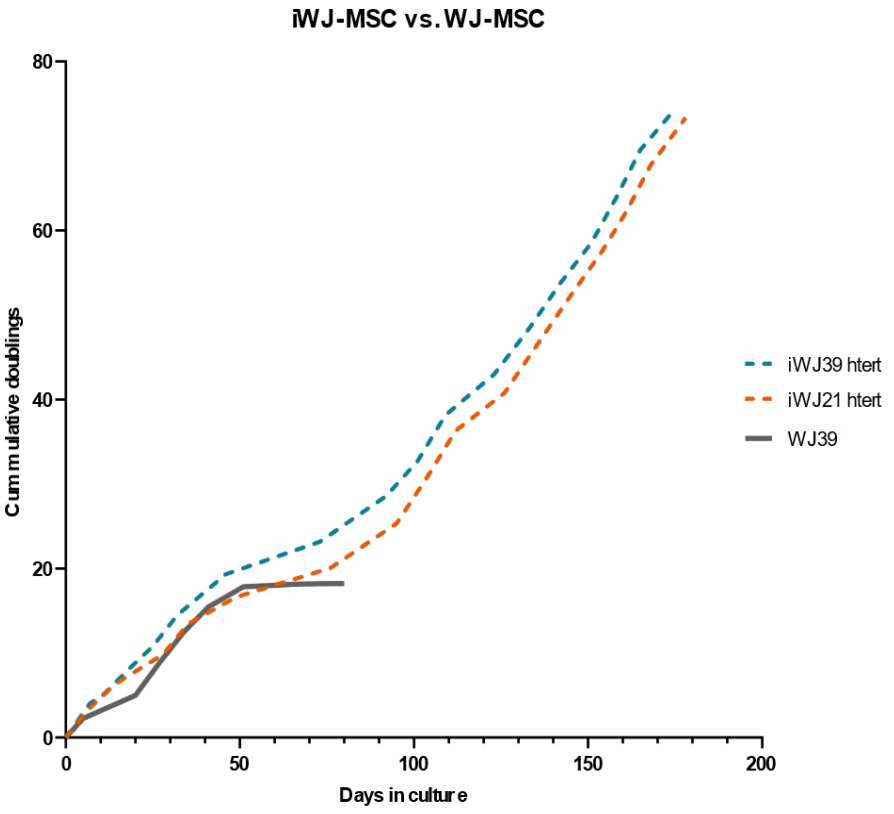
Background: Mesenchymal stromal Cells (MSC) are of particular interest in solid organ transplantation due to immunomodulatory and antifibrotic functions. MSC mechanism of action is not completely known, but *in vitro* and *in vivo* research highlighted the importance of MSC secretion products, especially extracellular vesicles (EVs or MSC-EVs). MSC-EVs as therapeutic agents possess several advantages over cellular therapies, including low responsiveness to the microenvironment, fewer adverse effects, lower immunogenicity and easy handling. Owing to these advantages, MSC-EVs are proposed as therapeutic tools in transplantation, however bulk GMP compliant production is still a challenge.

In this work, we aim to create an immortalized MSC cell line to generate and purify MSC-EVs by size exclusion chromatography (SEC) in a scalable and clinically translational manner.

Methods: To produce the MSC cell lines we immortalized Wharton's Jelly MSC (iWJ-MSC) transfected with hTERT. iWJ-MSC were cultured in 10% FBS αMEM medium to analyse doubling time stability and compare MSC characteristics. iWJ-MSC-EVs were isolated by SEC from conditioned medium in culture flasks. Furthermore, we used hollow fiber bioreactors to 3D-culture immortalized MSC in synthetic xeno-free medium and isolate iWJ-MSC-EVs by SEC.

Results: iWJ-MSC were comparable to primary cultures in surface markers, multilineage differentiation and PBMC derived T cell suppression capabilities while presented unlimited proliferation capacity until day 175. iWJ-MSC-EVs from primary and immortalized cell cultures suppressed T cell proliferation, and did not differ in any of the EV features analysed. Thirty million iWJ-MSC were cultured for 5 weeks in a hollow fiber bioreactor. Glucose consumption was low but stable and we did not observe significant cell death.

Conclusions: MSC-EVs can be generated and isolated from immortalized cell cultures with no significant alterations in standard cell and EV parameters. In addition, iWJ-MSC-EV production is scalable for downstream clinical application by culturing MSC in commercially available bioreactors clinically translational manner.



Doubling time (Day)	P(n)	P5	P10	P15
iWJ39 hTERT	3,03	2,38	3,34	2,83
iWJ21 hTERT	3,25	2,89	3,79	2,96

***Figure* 1:** iWJ-MSC Growth rate is expressed as cumulative doublings. iWJ-MSC kept their growth rate during follow-up period compared to the non-immortalized counterpart's growth rate of the four iWJ-MSC.