## OP212 THE MATRIX RELOADED: USING INTRAHEPATIC CHOLANGIOCYTE ORGANOIDS AND DECELLULARIZED HUMAN LIVER EXTRACELLULAR MATRIX TO CREATE FUNCTIONAL LIVER TISSUE

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**Background:** Liver transplantation is the only durable treatment for end-stage liver disease, but shortage of donor organs remains a limiting factor. Tissue engineered liver constructs could aid in decreasing donor shortage. A particularly promising approach is to repopulate decellularized human liver extracellular matrix (ECM) with patient-derived hepatobiliary stem cells. Intrahepatic cholangiocyte organoids (ICO) are an interesting cell source, as they can differentiate towards hepatocytes and cholangiocytes. Our aim is to determine the repopulation potential of ICO in decellularized liver ECM.
**Methods:** Human livers deemed unsuitable for transplantation (N = 3) were decellularized using a standardized protocol by pressure-controlled machine perfusion with 4% Triton-X-100 + 1% ammonia. No cells were found in the remaining extracellular matrix (ECM). Circular sections (Ø8 mm, thickness: 200 μm) were cut. ICO were initiated from healthy liver biopsies (N = 8) and expanded. The organoids were dissociated and added to the ECM sections as single cell suspension ($25 \cdot 10^4$ cells/section). The constructs were cultured in expansion medium or in hepatocyte differentiation medium. Cultures were terminated after 21 days and analyzed.
**Results:** ICO (N = 4) efficiently repopulated the liver ECM sections. It took 4–7 days for the cells to grow confluent layers on top of the ECM. During the following days, the cells self-organized into columnar shaped cells as shown by histological analysis. KRT-7 (cholangiocyte) and albumin (hepatocyte) markers increased 4-fold (SD: ±7) and 17-fold (SD: ±26) respectively compared to controls. Albumin expression increased 35-fold (SD: ±36) and LGR5 (stem cell marker) decreased 8-fold (SD: ±4) after differentiation. Albumin and KRT-7 were also detectable with immunofluorescence.
**Conclusion:** ICO are capable of repopulating decellularized human liver ECM segments *in vitro*. The ECM appears to drive self-differentiation of the ICO towards cholangiocytes and hepatocytes as marker increase when cultured in expansion or hepatocyte differentiation medium. These results encourage upscaling towards larger-scale perfusion based recellularization experiments where patient-derived ICO can be used to engineer functional and clinically relevant hepatic tissue *in vitro*.

## OP213 PERFUSION-DECELLULARIZATION OF VASCULARIZED BONE MATRIX: APPLICATION TO THE PORCINE FORELIMB

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**Background:** Large bone defects still remain a challenge for the surgeon, and available reimplanted bone substitutes can't properly restore optimal function along with long term osteointegration and full survival of the bone graft. We present in this study a new animal model of bone substitute based on the perfusion-decellularization technique, applied on vascularized porcine bone shafts xenografts.
**Methods:** 11 porcine bone forelimbs including the radius and the ulna were harvested with their vasculature based on an interosseous artery and perfusion-decellularized. Characterization of the obtained matrices was performed through histological analyses, DNA and ECM proteins dosages, density acquisitions and biomechanical testing. The quality and preservation of the vasculature was assessed through barium-sulfate injected CBCT acquisitions. The potential for in vivo reimplantation was assessed through fibroblast static cell seeding, adipose mesenchymal stem (AMS) cells seeding and evaluated with classic histological staining and immunohistochemistry.
**Results**: Decellularization was successful for all grafts with excellent preservation of the ECM and global structure. DNA and ECM proteins measurements revealed optimal clearing of the cellular compartment and preservation of major proteins. Density acquisitions revealed a slight decrease of density whereas biomechanical testing was unmodified. The vasculature was entirely preserved throughout the whole graft and all seeded cells were viable in all samples, with the initiating of new bone formation and osteogenic differentiation of AMS-cells.
**Conclusions:** Fully vascularized decellularized and transplantable bone shafts xenografts were obtained with true potential for in-vivo reimplantation. Thereby, they may offer in a close future new perspective for large bone defects repair, and in global bone tissue engineering.

## OP214 ANTI-FIBROTIC EFFECTS OF MEMBRANE PARTICLES FROM MESENCHYMAL STROMAL CELLS IN A RENAL ISCHEMIA REPERFUSION INJURY MOUSE MODEL

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**Background:** Membrane particles (MP) are nanovesicles artificially generated by extrusion of the Mesenchymal Stromal Cell (MSC) membranes. MP were designed to circumvent the risks of MSC therapy such as a poor biodistribution due to their large size and unknown behaviour after infusion, while keeping the reparative properties of MSC. We demonstrated earlier that MP have endothelial regenerative capacity, and antifibrotic effect on lung fibroblasts. In this study, the aim is to explore whether MP have antifibrotic effect on a renal ischemia reperfusion injury (IRI) mouse model.
**Methods:** IRI was performed by clamping the right kidney of the mice for 37 min. The MP were intravenously infused 3–5 h after ischemia. The kidneys were harvested 3 days after renal IRI. Four groups of mice were analysed: Sham, IRI, IRI + MP derived from 1 million of MSC, and IRI + MP derived from half million of MSC. Gene expression of proinflammatory cytokines was measured in the kidneys such as IL6, and TNFα; and kidney injury marker KIM-1; and profibrotic molecules such as TGFβ, PAI-1, fibronectin, collagen I, and III. Sirius red for collagen staining was performed to evaluate fibrosis in the histological analysis of the kidneys.
**Results**: We found no difference between IRI mice treated with MP and IRI untreated mice with respect to the proinflammatory markers. IRI induced an upregulation of the mRNA expression of profibrotic genes such as TGFβ and PAI-1, and proteins from the extracellular matrix, including fibronectin, collagen I, and III. Interestingly, the higher doses of MP significantly decreased the expression of TGFβ, PAI-1 and the main extracellular matrix proteins involved in fibrogenesis. The percentage of fibrosis area in the histological analysis of the kidneys were in line with the results observed in the gene expression of profibrotic markers.
**Conclusions:** Our findings show that MP have early antifibrotic effects on renal IRI. Future research will address questions including mechanisms of action and anti-fibrotic effects in long term with the goal of using MP as a new approach to combat renal fibrosis.

### BIOMARKERS: WHAT'S CIRCULATING?

## OP215 IMPACT OF DELAYED GRAFT FUNCTION ON BASELINE DONOR DERIVED CELL-FREE DNA (DD-CFDNA) IN KIDNEY TRANSPLANT RECIPIENTS

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**Background:** Donor derived-cell free DNA (dd-cfDNA) is a serum biomarker now available to predict acute rejection in renal allografts. The technology utilizes targeted next generation sequencing and does not require donor genotyping. A level ≥1% suggests allograft injury usually from acute rejection. Delayed graft function (DGF) can be associated with ongoing allograft inflammation. We aimed to evaluate the impact of DGF on baseline dd-cfDNA levels in kidney transplant recipients (KTRs).
**Methods:** Our center has been checking dd-cfDNA levels (AlloSure, CareDx, Brisbane, CA) as for-cause and surveillance in high immunological risk KTRs since 2018. We identified patients who underwent deceased donor kidney transplantation at our center between April 2018 and June 2020 who