MEETING Belgian Transplantation Society 28-03-2025 (Sparks, Brussels)

ABSTRACT SUBMISSION FORM

Submission deadline: 31/12/2024

(for office use only)

PART I: Abstract Box

Title: Modelling Ischemic Cholangiopathy: Exploring Primary Human Cholangiocyte Organoids as a Translational Research Tool.

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Background: One-third of liver transplant recipients will develop ischemic cholangiopathy (IC) within the 1st year post-transplant, with 50% requiring re-transplantation. Donation after circulatory death (DCD) increases the risk of IC by 11-fold, but our limited understanding of cholangiocyte ischemia-reperfusion injury impedes IC prevention and treatment. We aimed to explore human cholangiocyte organoids (CO) as a preclinical model to investigate the pathophysiology of cholangiocyte ischemia-reperfusion injury, by comparing COs derived from donation after brain death (DBD) and DCD livers.

Methods: The gallbladder epithelium from DBD (n=2) and DCD (n=2) livers was mechanically dissociated. Primary cholangiocytes were cultured in matrigel with R-spondin, Epithelial Growth Factor, Dickkopf-related protein 1, and Y-27632. The development of organoids was then followed daily for a month by live-cell imaging. RT-qPCR was used to confirm the expression of cholangiocellular markers cytokeratin 7 (KRT7) and 19 (KRT19), gamma-glutamyl transferase 1 (GGT1), and the SRY-Box Transcription Factor 9 (SOX9).

Results: CO were visible in bright field imaging as hollow spheres with cells lining a clear lumen from the first culturing day. In the following days, COs expanded, divided, propagated, and reached confluence within **6 (±1) days (Figure 1).** RT-qPCR confirmed the expression of cholangiocyte-specific marker genes KRT7, KRT19, GGT1, and SOX9 in COs, regardless of donor type. Live-cell imaging showed significantly fewer COs from DCD than DBD donors (**p=0.001**) at passage 0 **(Figure 2).** While organoid numbers increased significantly in both groups over time (**p<0.0001**), DCD-derived COs exhibited slower growth rates (**p<0.0001**). This difference was not visible at passages 1 and 3. COs size was similar.

Conclusions: We successfully generated COs with both stemness and mature cholangiocyte profiles. Importantly, our preliminary results suggest that this COs retain donation-induced injury traits. Further expansion of the sample size, thorough characterization, and functional testing necessary to further validate this model are in progress.

Part II: Administrative data

A. Topic categories (check one)

☐ Experimental ☐ Immunobiology ☐ Basic science

☐ Heart-lung ☒ Liver-Intestine ☐ Kidney-pancreas

☐ Organ allocation/procurement/ethics

B. Acceptance policy (Check box)

☒ I certify that 1) this abstract meets the abstract submission guidelines, 2) I accept the abstract policies and procedures on the abstract submission guidelines, and 3) All authors are familiar with the presented data.

C. Name of the presenting author

Name:Dos Santos Barata Available day of congress: ☒ Yes ☐ No

First name: Elisa

D. Presenting author (Check box)

☒ BTS member ☐ Non BTS member

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☒ Yes ☐ No

Part III: Additional Abstract Figures/Tables (Maximum 2 in total)

Table/Figure 1

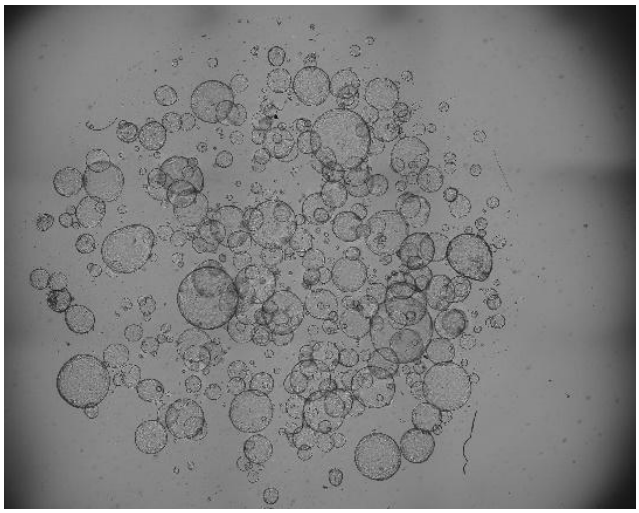
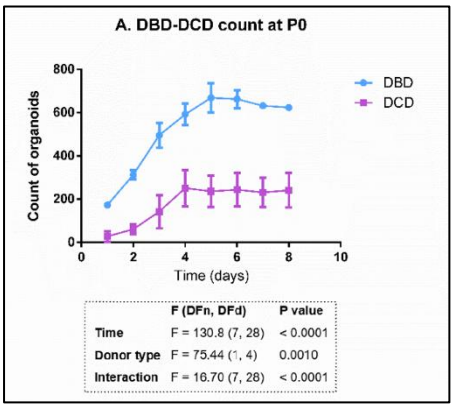


Figure legend 1: Bright field image of cholangiocyte organoids.

Bright-field images taken with the Incucyte SX5 Imaging System (Sartorius) with a 4x magnification.

Figure and Table 2



B.

Culturing day P0	Average CO number (SD) - DBD	Average CO number (SD) - DCD
1	173 (11.79)	27.333 (24.826)
2	313 (21.071)	60.667 (22.502)
3	495.667 (57.274)	142.333 (76.957)
4	592 (49.689)	251 (84.285)
5	669 (67.55)	236 (72.187)
6	663.333 (42.36)	243.667 (77.054)
7	632 (7.81)	230.667 (67.278)
8	624 (7.55)	240.667 (79.431)

Figure/table legend 2: Graph (**2A**) and table (**2B**) representing the averaged organoid count of DBD and DCD organoids culture replicates (n=3) at P0.

Abbreviations 2: DBD, donation after brain death; DCD, donation after circulatory death; P0, passage 0; CO, cholangiocyte organoid; SD, standard deviation