**Introduction**

Tissue architecture and hepatic cell morphology reflect the functional differentiation of the liver. Differentiated functions of hepatocytes, depend on complex, heterotypic cell-cell/cell-matrix interactions mediated by cell adhesion molecules (CAMs) in a 3D microenvironment. Vascularisation of human hepatic tissue for therapeutic/pharmaceutical applications, requires knowledge of optimal trophic conditions to support different cell types and how these cells behave embedded in different biocompatible matrices.

**Methods**

The authors aimed to assess morphology, metabolic functionality and phenotypic expression of human progenitor EoCs (Endothelial outgrowth Cells) vascular microvessels, cocultured with hepatic C3A cells (C3As), EoC/C3A cocultures in different media and test biomatrices: Matrigel/MaxGel/ Puramatrix (self-assembling nanofibres); and control standard 2D cultures (tissue culture plastic; TCP) were analysed following immunostaining using morphology (light/confocal microscopy); and flow cytometry. Metabolomics analysis of culture media provided a global picture of metabolic changes in response to hepatic/EoC coculture (vs reference C3A monocultures).

**Results**

Titration in standard 2D/TCP co-culture in various media showed a ratio of 3C3A:1EoC in Lonza EGM-2 medium was optimal. Cells retained phenotypic expression of differentiation markers: (1) Hepatic: Albumin, EpCAM, E-CAD; (2) EoC: CD146, CD31, CD105, VWF; as evidenced by flow cytometry/immunostaining. Metabolomics analysis of media (from 2D/TCP co-cultures), showed modulation of key hepatic metabolic intermediaries including: (1) Urea cycle: 50% enhancement of L-ornithine production; (2) Biosynthesis: Bile Acids: Enhanced Glycocholate; amino acid (eg, Taurine) utilisation; and Creatine production: (3) Antioxidants: co-culture ameliorated the requirement of high Glutathione antioxidant levels inherent to EoC monocultures. Test biomatrices under different 3D culture configurations, showed: (1) MaxGel sandwich culture promoted differentiated (cuboidal) morphology of C3As, but not EoCs; (2) Conversely, only EoCs overlaid on MaxGel formed differentiated (microvessel) structures; (3) Puramatrix supported 3D culture of C3As but not EoCs; (4) Matrigel supported only EoCs 3D microtubular structures.

**Conclusion**

This study proved informative for engineering of vascularised organoids for future clinical/pharmaceutical applications. Work in progress include comprehensive metabolomics analysis, flow cytometric profiling and combinatorial analysis of cell-supportive biomatrix configurations to further optimise 3D coculture.

**Competing interests**

None.

**Keywords**

co-culture, endothelial progenitors, liver cell line, liver organoids.