

Production and characterization of human liver extracellular matrix hydrogels for *in vitro* culture of distinct human primary liver cell populations.

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Background and Aims: The study of human primary liver cell biology is largely hampered by the lack of adequate *in vitro* culture systems that allow the maintenance of the cells' viability and physiologic phenotype for prolonged periods of time. In this context, a growing interest for liver decellularization and for the use of liver extracellular matrix (ECM) in cell culture and tissue engineering applications emerged over the past five years. Whereas several publications describe detailed procedures for animal liver decellularization and ECM-based hydrogel production, few is known about the composition and properties of isolated human liver ECM. In this study, we analyzed the composition of human liver ECM and derived hydrogels, and we further addressed the question of the potential benefits of a liver ECM protein-coated surface for the culture of primary human liver cells.

Method: A combination of thermic and osmotic shock, enzymatic digestion and detergents has been used to decellularize human liver fragments unsuitable for transplantation. Liver ECM was partially digested with pepsin for coating purposes and characterized by histology and mass spectrometry. Primary human liver cells, including hepatocytes, hepatic stellate cells (HSC) and endothelial cells (EC) were seeded on liver-coated culture dishes. Adhesion (2h), viability (24h), proliferation (7 days) and phenotypic properties were evaluated and compared to a surface of reference (CellBIND or rat tail collagen coating).

Results: Our protocol led to a residual amount dsDNA below decellularization standards. Coating derived from liver ECM showed no toxicity for primary human liver cells. Mass spectrometry revealed the abundance of type I and type III fibrillar collagen components (COL1A1, COL1A2, COL3A1) as well as elastin in liver ECM hydrogels used for coating. Preliminary results further suggest the benefits of human liver ECM on human primary liver cell properties in 2D culture as compared to surfaces of reference.

Conclusion: Our study provides a first detailed characterization of human liver ECM and derived hydrogels, easily and reproducibly isolated from small human liver fragments. Our results further open the way for the use of human liver ECM as a coating for the culture of primary human liver cells in 2D. In a near future, 3D applications will be developed.

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