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

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1. INTRODUCTION

- 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.
- There is a growing interest in liver-specific extracellular matrix (ECM) for cell culture and tissue engineering applications [1,2].

2. AIM

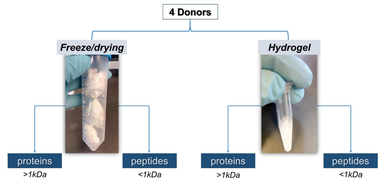
In this study, we analyze the composition of human liver-specific ECM and derived hydrogels, and we further address the question of the potential benefits of a liver ECM protein-coated surface for the culture of primary human liver cells.

3. METHODS

Liver decellularization process

❑ A combination of mechanical, chemical and enzymatic methods have been used in order to decellularize human liver fragments, unsuitable for transplantation, from four healthy donors [1,2].

Proteomic analysis of decellularized liver ECM



❑ Liver ECM was partially digested with pepsin [1] for coating purposes and characterized by histology and mass spectrometry after acetone precipitation and peptides and proteins separation.

❑ Human liver cells were isolated using a two-step perfusion technique, as previously described [3].

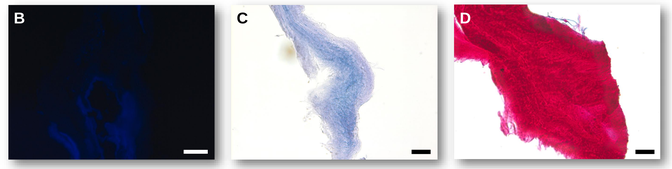
4. RESULTS

I. Evaluation of the decellularized liver ECM

A **DNA content**

Donor	dsDNA (ng/mg of ECM)
154	9,0
155	19,6

< Max 50 ng/mg ECM



*(**A**) dsDNA quantification demonstrates good cellular and nuclei material removal. These results are confirmed by (**B**) DAPI and (**C**) Masson's Trichrome staining. (**C**) Masson's Trichrome and (**D**) Sirius Red staining are indicative of the collagen content. The pictures were taken at 20X (B) and 40X (C, D) magnification and the scale bar corresponds to 50 µm (B) and 20µm (C,D).*

II. Liver ECM hydrogel proteomic characterization

A Hydrogel Peptidomics

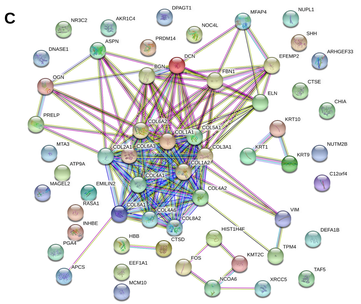
Protein	Abundance Score	Donor 1	Donor 2	Donor 3	Donor 4
Elastin (GN=ELN)	3352,42	High	High	High	High
Collagen alpha-2(I) chain (GN=COL1A2)	1894,33	High	High	High	High
Collagen alpha-3(VI) chain (GN=COL6A3)	351,5	High	High	High	High
Collagen alpha-1(I) chain (GN=COL1A1)	947,59	High	High	High	High
Collagen alpha-1(V) chain (GN=COL5A1)	150,71	Not Found	High	High	High
Collagen alpha-6(IV) chain (GN=COL4A6)	25,23	Not Found	High	High	High

B Hydrogel Proteomics

Protein	Abundance Score	Donor 1	Donor 2	Donor 3	Donor 4
Collagen alpha-2(I) chain (GN=COL1A2)	1894,33	High	High	High	High
Collagen alpha-1(I) chain (GN=COL1A1)	947,59	High	High	High	High
Pepsin A-4 (GN=PGA4)	202,72	High	High	High	High
Collagen alpha-1(V) (GN=COL5A1)	150,71	Not Found	High	High	High
Collagen alpha-1(III) chain (GN=COL3A1)	53,33	Not Found	High	High	High
Collagen alpha-1(II) chain (GN=COL2A1)	53,47	Not Found	High	High	High
Collagen alpha-6(IV) chain (GN=COL4A6)	25,23	Not Found	High	High	High

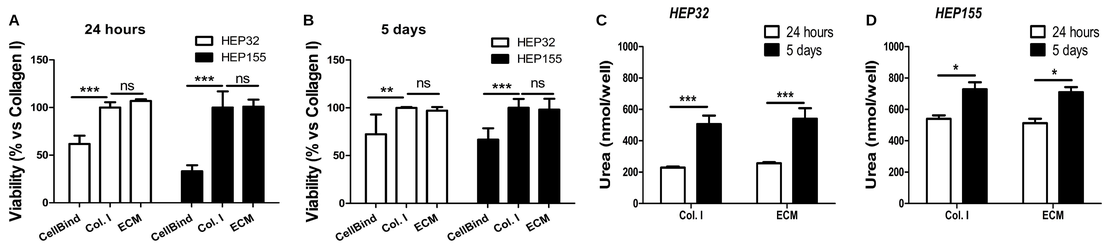
- ***261 different proteins identified in the ECM***
- ***59 different proteins identified in the hydrogels***

C



*In all donors hydrogels we identified 12 types of collagens. Tables are showing the most abundant peptides (**A**) and proteins (**B**) found in the ECM hydrogel produced from each donor. The illustration (**C**) shows the interaction network of the different proteins identified in the hydrogels.*

III. Liver ECM hydrogel in vitro evaluation with primary human hepatocytes



*Human hepatocytes (HEP) cultured on a liver ECM derived hydrogel coated surface. (**A**) Viability of HEP on CellBind © (negative control), rat tail collagen I coating (positive control), and human liver ECM coating after 24h of culture. (**B**) Viability of HEP on the same surfaces after 5 days of culture. (**C** & **D**) Urea production by HEP in cell culture medium after 24h or 5 days of culture on rat tail collagen I coating and human liver ECM coating. All presented data have been produced using human hepatocytes from donors F32 (n=5) and F155 (n=5), ns $p > 0.05$, *** $p < 0.005$, * $p < 0.05$.*

5. CONCLUSIONS

- We are able to efficiently produce decellularized human liver-derived ECM.
- There are qualitative and quantitative differences in the proteins contained in the ECM of each donor.
- The majority of the identified proteins contained in the ECM consists of structural proteins.
- Liver ECM-derived hydrogels can be used to coat plastic surface and is suitable for liver cell culture.
- Human liver ECM coating preserves human hepatocytes viability and urea synthesis at the same levels as rat tail collagen type I coating.

❖ **Perspectives:** We are currently performing additional functional studies on human hepatocytes and other human primary liver cells (hepatic stellate cells and liver endothelial cells) cultured on liver ECM derived hydrogels

6. REFERENCES

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7. ACKNOWLEDGEMENTS

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