MIMICKING LIVER MICROENVIRONMENT IN A 3D *IN VITRO* MODEL: COLLAGEN HYDROGELS FUNCTIONALIZED WITH FIBRONECTIN

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Introduction

Current liver *in vitro* models exhibit numerous limitations and low similarity with the native organ, as they fail in the replication of the 3D microstructure and composition found *in vivo*. Therefore, the design of three-dimensional (3D) models that mimic the liver matrix and cell-matrix interactions remains to be explored to finally unseat 2D cultures [1]. This study aims to address this gap by proposing a 3D culture platform for hepatic cells based on collagen (Col) hydrogels functionalized with fibronectin (FN). Our model is inspired in the natural matrix: collagen as the main component of the liver matrix and FN as an important biomolecule in cell-cell communication and hepatic cell differentiation [2,3,4]. The main objective of the study is to evaluate the role of FN in the culture of HepaRG cells by assessing the functionality of cells compared to 2D cultures (*Fig. 1*). The final goal will be to use the improved 3D system as a drug screening platform.

Methods

Mechanical and physicochemical characterization. Mechanical properties were determined by rheology and equilibrium water content was also calculated in Col and ColFN with different ratios to finally select the optimal one. Fibronectin was physically incorporated in hydrogels and its retention was assessed by ELISA.

HepaRG cells encapsulation and liver functionality. Cells were encapsulated in Col and ColFN hydrogels, differentiated using 1% of DMSO and compared to monolayers differentiated with the standard protocol (2% DMSO).

The effect of 3D culture on liver functionality was tested. LIVE/DEAD, albumin and urea production were evaluated. Changes in the gene expression of key hepatic markers were tested by qPCR and the expression of hepatic proteins (albumin) was checked by immunofluorescence.

Results

Hydrogels showed tailored mechanical properties with storage modulus values near the liver tissue (600 Pa) and fast gelation times (10 min). Col-FN hydrogels did not show significant changes in their properties. HepaRG cells were alive (90% viability) after 14 days of culture in 3D hydrogels and showed increased urea and albumin production compared to the 2D. Gene expression revealed significant changes in the expression of genes coding for enzymes important in drug detoxification and liver-specific genes such as albumin in 3D compared to 2D. Encapsulated cells were organized in 3D clusters and expressed liver proteins such as albumin and factor HNF4α (*Fig. 1*).

Discussion

The results demonstrated that the 3D enhanced the liver functionality of encapsulated cells compared with the 2D in less time and DMSO concentration. These hydrogels could be considered as a 3D liver culture platform to replace conventional 2D models with significant potential to be explored.

Figure and Tables

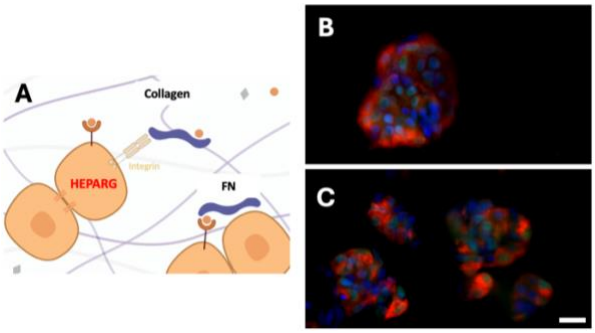

Figure 1. (A) Graphic scheme of the proposed model. (B) Immunofluorescence images of, HNF4α (green) and albumin (red) expression in cells encapsulated in Col and (C) ColFN. Nuclei were stained with Hoechst 33342 (blue).

References

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