VISCOELASTIC HYDROGELS AS 3D PLATFORMS FOR CELL CULTURE

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Introduction

Different approaches have been developed during the last years to generate 3D *in vitro* liver models that replicate the *in vivo* microenvironment to facilitate the understanding of hepatocytes functionality and their alterations in various pathologies. The use of biomaterials naturally found in the liver extracellular matrix (ECM) to generate hydrogels is an interesting approach that promotes cell-cell communication and cell-matrix interactions that resemble the *in vivo* situation (1). Despite different hydrogels have been developed for hepatic disease modeling, only few of them consider how the dynamics in the mechanical properties of the environment influences cell response (2). Most of them devote cell response to substrate stiffness, but do not consider that the liver is a viscoelastic organ in which the cellular response to mechanical stimuli from the ECM depends on the time and frequency of the stimulus (3). This study aims to generate novel *in vitro* models of healthy liver by engineering viscoelastic hydrogels with reversible bonds for the future 3D culture of hepatic cells.

Methods

Synthesis of viscoelastic hydrogels: dual functionalized gelatin (Gel) with norbonene and boric acid (Gel-NB-BA) (8% wt/vol) was mixed with dopamine-functionalized hyaluronic acid (HA) (HA-DOPA) (1,5% wt/vol) in a 90:10 ratio. The final hydrogels were covalently crosslinked due to the thiol-norbonene gelatin crosslinking with PEG4SH during 30 minutes under 365 nm UV light exposure with 10 mM lithium phenyl 2,4,6 trimethyl as photoinitiator. The BA-DOPA interactions by reversible bonding provided the viscoelastic properties.

Grafting analysis: the grafting analysis of the different functional groups bonded to Gel (NB and BA) and HA (DOPA) was confirmed by spectrophotometry.

Mechanical characterization: mechanical properties were determined by rheology. Pieces of fresh pig liver were measured, and the Gel-NB-BA/HA-DOPA hydrogels proportion was adapted to mimic the mechanical properties of the liver.

Cytotoxicity tests: as a first approach to test the cytotoxicity of the hydrogels, L929 murine fibroblasts were encapsulated and cell viability was assessed by a live/dead assay.

Results and discussion

Grafting analysis corroborated the incorporation of the different functional groups within the hydrogels precursors (NB, BA, DOPA). The Gel-NB-BA/HA-DOPA hydrogels display viscoelastic properties due to the reversible bonding between BA-DOPA groups in the presence of water (4,5). After assessing the mechanical properties of healthy pig livers by rheology, the hydrogels' proportion was adapted to imitate the biomechanical environment of hepatic tissue (*Figure 1*). Live/dead results proved that UV exposure did not produce any effect on cell viability after 30 minutes of hydrogel crosslinking. These results confirm that the viscoelastic hydrogels are 3D systems with potential applications in liver tissue engineering.

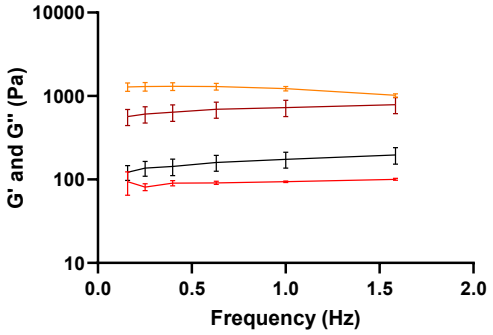

Figure 1: Rheological measurements of Gel-NB-BA/HA-DOPA hydrogels compared with pig liver. G' (orange for hydrogels and dark red for liver) and G'' (black for liver and red for hydrogels) values are represented.

References

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Acknowledgements

Supported by PID2022-136433OB-C21, -C22 / AEI/10.13039/501100011033 and CNS2022-135425 grants from the Spanish State Research Agency and PI21/00223 grant form Institute of Health Carlos III. Also supported by Generalitat Valenciana through CIPROM/2022/43 project.