The Effect of Matrix Stiffness on Lipid Processing, Cell Function, and Morphology in HepG2 cells

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INTRODUCTION

- Disease progression to cirrhosis in Non-Alcoholic Steatohepatitis (NASH) leads to increased mechanical stiffness that affects liver parenchymal and non-parenchymal cells.
- Mechanical stiffness of the extracellular matrix (ECM) is sensed by cells through mechanotransduction, which influences cell function and metabolism.

AIM

To model how liver ECM stiffness affects liver specific HepG2 cell lipid processing, cell function and morphology.

MATERIALS AND METHODS

- Polyacrylamide gels of three different stiffnesses, 0.1 kPa, 1.3 kPa, and 35 kPa were cast and covered with Collagen I.
- HepG2 cells were treated with serum-free media or supplemented with 200 uM Oleic Acid (OA) for 24 hours on collagen laden gels.
- Cells were imaged using Bodipy neutral lipid stain, Hoechst nuclei stain, and Phalloidin stain for the actin cytoskeleton.
- Cell analysis was performed using ImageJ.
- Immunofluorescence assessed the nuclear transcription factors YAP1 and HNF4-a.
- qPCR and RNAseq evaluated gene expression.

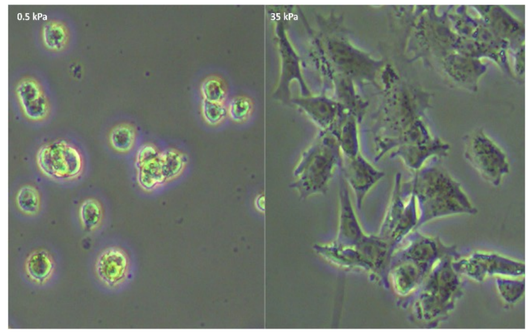
RESULTS

Morphology:
- Increased (Inc) Cell Spread (avg cell area: 146 uM and 277 uM in 0.1 and 35 kPa)

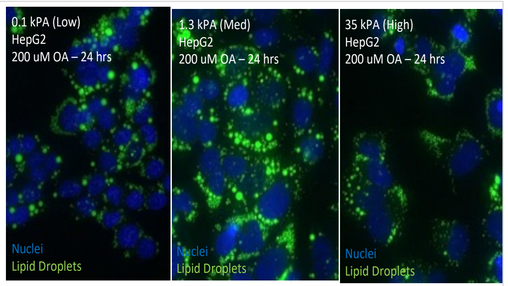
Lipid Processing (200 uM OA)*:*
- Inc microvesicular steatosis (avg lipid droplet size: 1.64 uM and 0.565 uM in 0.1 kPa and 35 kPa, p<0.01)

Immunofluorescence*:*
- Inc YAP1 nuclear localization, Dec HNF4-a (nuclear/cytoplasmic ratio YAP1: 0.40 and 0.55 in 0.1 kPa and 35 kPa, p<0.01; HNF4-a: 0.82 and 0.54 in 0.1 and 35 kPa, p<0.05)

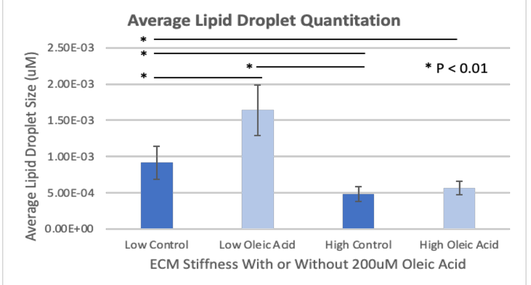
RNAseq *(200uM OA 35 kPa vs. 0.1 kPa):*
- Inc mitochondrial metabolic gene expression MT-ND1, MT-ND2, MT-CO2, MT-ATP8, MT-ATP6 (p<0.05).
- Inc mechanotransduction and membrane rigidity gene expression, HLA-F, MATN3, and TLCD2 (p<0.01).



Above, Left: HepG2 cell morphology on low stiffness (0.5 kPa) gels. **Above, Right:** HepG2 cell morphology on high Stiffness (35 kPa) gels.



Above Left: HepG2 cell staining of lipid droplet with bodipy (green) and nuclei with Hoescht (blue) on gel scaffolds of different stiffnesses after 24 hours of 200 uM oleic acid treatment.



Above Right: Quantitation of the average lipid droplet size in HepG2 cells that were either grown in control conditions or subject to 24 hours of oleic acid treatment.

CONCLUSION

- Increasing matrix stiffness affects lipid processing, transcription factor gene expression, and cell morphology of HepG2 cells.
- This reflects the changes that cells experience throughout disease progression through fibrosis and cirrhosis, altering gene expression, structure, and function.

ACKNOWLEDGEMENTS

T32 DK077662
R01 DK093807
We would like to acknowledge the McCormick Foundation and their contribution through the McCormick Grant.

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DISCLOSURES

Nothing to disclose

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