Summary of: Molecular mechanisms underlying the enhanced functions of three-dimensional hepatocyte aggregates.pdf

Key findings and quantitative results:

1. **Improved Functions in 3D Culture**: - **Synthetic and Metabolic Functions**: Primary mouse hepatocytes cultured in RWVs demonstrated significantly higher albumin secretion and cytochrome P450 1a1 (CYP1A1) activity compared to those cultured in TCDs at both early and late time points. - **Gene Expression**: Global gene expression analysis revealed that genes upregulated in 3D culture were distinct from those upregulated during liver development and regeneration. - **Hnf4a Expression**: Hnf4a expression was significantly increased in RWV cultures compared to TCDs, with Hnf4a TFBSs in the promoters of many genes upregulated in RWV cultures.

2. **Comparison to Monolayer Cultures**: - **Monolayer Cultures**: Expression of proliferating cell nuclear antigen (Pcna) was higher in TCDs than in RWVs at 36 hours of culture. - **Mesenchymal and Cytoskeletal Genes**: Expression of mesenchymal and cytoskeletal genes was suppressed in RWV cultures compared to TCDs.

3. **Promoter Region Analysis**: - **Over-represented TFBS**: oPOSSOM identified HNF4α as the most significantly over-represented TFBS in genes upregulated in RWV cultures. - **Z-score and Fisher Score**: The Z-score and Fisher score were used to validate the TFBSs, with HNF4α having a high Z-score and Fisher score.

4. **Consequences of Monolayer vs. 3D Culture**: - **Mesenchymal Transition**: Expression of mesenchymal genes was significantly higher in TCD cultures compared to RWVs. - **Cytoskeletal Genes**: Several cytoskeletal genes not previously described in monolayer culture were significantly upregulated in TCD cultures compared to RWVs.

5. **Implications**: - **Hnf4a Role**: Hnf4a is a master regulator of hepatocyte functions, maintaining differentiated hepatocyte functions in 3D culture. - **Regulation**: Hnf4a represses mesenchymal genes, leading to a mesenchymal-to-epithelial transition in TCD cultures.

6. **Conclusion**: - **3D Culture Benefits**: 3D cell-cell interactions are crucial for maintaining Hnf4a expression, which in turn sustains hepatocyte-specific functions. - **Optimization**: Biomaterials should maximize 3D cell-cell interactions to optimize hepatocyte functions.

These findings highlight the importance of 3D cell-cell interactions in maintaining hepatocyte functions, providing a foundation for rational design of biomaterials for biomedical applications.