3D bioprinting as an alternative to traditional animal testing models: advancing the incorporation of immune cells

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Abstract

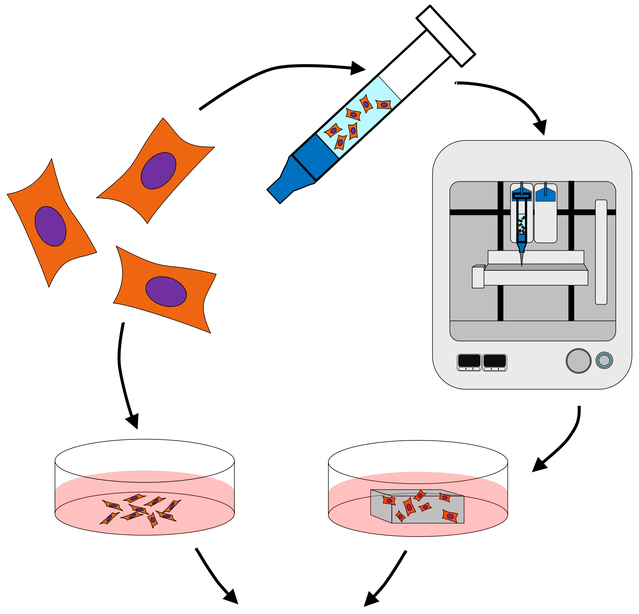
The NRC Biomedical Nanotechnology team is seeking to improve its expertise and infrastructure for 3D bioprinting. 3D bioprinting is an emerging technology that has the potential for many biomedical and therapeutic applications. Currently, several organizations, including the US food and drug administration, have expressed interest in utilizing alternatives to animal models, such as organoids, organs-on-chips and 3D printed tissues. Construction of 3D printed tissues that can accurately mimic the complex interplay between various layers of tissues and cell types would offer a potential platform for drug and chemical testing. Despite much progress in the last decade, 3D bioprinting has seen limited use in clinical settings or drug development. One aspect that is often missing from 3D printed tissue is the inclusion of immune cells. This is particularly important for testing novel chemicals, as they may be relatively inert to most cells but could induce a significant response from immune cells. For this reason, we are investigating ways to incorporate immune cells into 3D printed matrices with epithelial cells. To assess whether our printing protocol could successfully incorporate epithelial cells, we printed a 3D matrix with A431 cells and tested gene expression against a 2D culture using a commercial epithelial cell qPCR array. We identified several changes in genes previously found to be associated with 3D culture, including genes associated with cell fate and proliferation.

Introduction

- There is significant interest in animal model alternatives [1].
- 3D printed tissues offer a promising alternative.
- For use in pre-clinical safety testing, it will be important that any 3D printed tissues contain the proper repertoire of immune cells [2].
- For this reason, our research group is looking into expanding our 3D bioprinting capacity with the intent to study methods for incorporation of immune cells into bioprinted matrices and tissues.
- Our group has previously printed immune cells into 3D matrices [3,4].
- In order to make a mixed population of epithelial and immune cells, we sought to incorporate epithelial cells into a 3D matrix and ensure cells exhibited the expected changes when cultured in 3D vs 2D..

Methods

- A human epithelial cell line (A431) was mixed with CELLINK Bioink, a commercial bioink composed of alginate and cellulose fibrils.
- 5X5X5 mm rectilinear constructs were then bioprinted into a 24 well plate and crosslinked with $CaCl_2$.
- Constructs were then washed and incubated in complete media for 24 hours at 37°C.
- In parallel, A431 cells were grown to 70-80% confluency in traditional 2D culture.
- RNA was extracted from both cultures using trizol and Qiagen RNeasy mini extraction columns. RNA quality was assessed using the Nanodrop One Spectrophotometer and Bio-rad Experion Bioanalyzer.
- cDNA was synthesized using 1 µg of total RNA and High capacity cDNA reverse transcription Kit. 20 ng of cDNA was utilized for setting up the qRT-PCR by utilizing a GeneQuery Human Epithelial Cell Biology qPCR array Kit.
- Fold gene expression was calculated using the $2^{\Delta\Delta CT}$ method and plotted using the R package ggplot2 [5].
- DAVID bioinformatics [6,7] was used to identify KEGG pathways and GO terms represented in the qPCR array and identify relevant genes.



Results

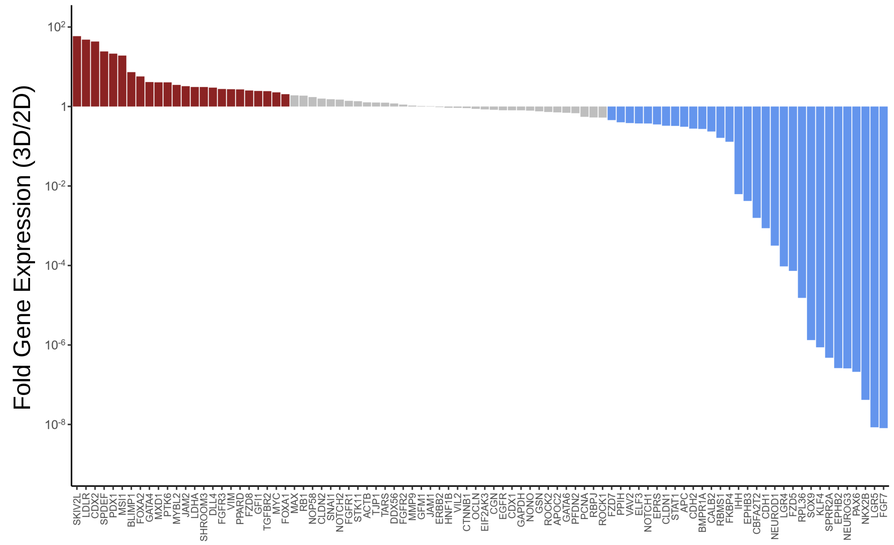


Figure 1 – Gene expression changes in A431 cells when cultured in 3D vs 2D
Changes in gene expression of A431 cells cultured in 3D and 2D culture as described in methods are presented as fold change of 3D vs 2D culture. Color of each bar represents those genes that increased (red) or decreased (blue) more than two fold in 3D culture.

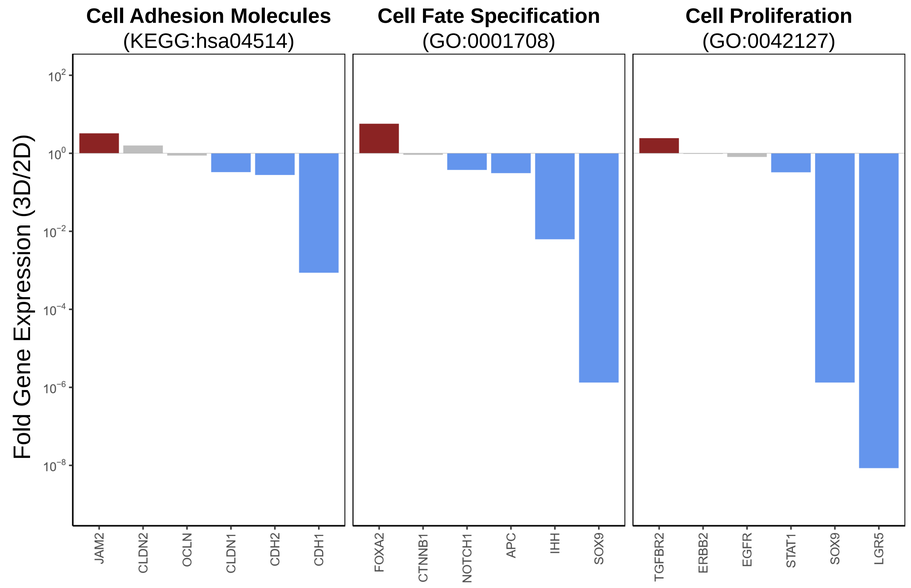


Figure 2 – Changes to selected pathways/gene ontology groupings.
Gene expression changes for specific genes from indicated KEGG pathway/ Gene ontology (GO) groupings are shown together.

Conclusion

- Culture of cells in 3D seemed to induce noticeable changes in gene expression as compared to cells in 2D.
- Many genes involved in cell fate and proliferation were downregulated, possibly due to slower initial growth previously seen in epithelial cells in 3D culture [8].
- Several genes in the cell adhesion molecules pathway were downregulated, as had been shown previously [8].
- More replicates will be required to determine whether these differences are statistically significant.

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Acknowledgments

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