Perfusion Bioreactor for 3D Cell Culture

Investigating Adipocyte Mechanobiology

Beatrice Bezdadea, Rares Dorcioman, Charalambos Hadjipanayi, Emmanuel Gkigkilinis, Georgios Gryparis

Imperial College London

Department of Bioengineering
Supervisor: Dr Darryl Overby

Introduction

- Adipocytes are mechanoresponsive and activate feedback pathways that modulate adipostasis [1].
- Cyclic stretch and static compression inhibit pre-adipocyte differentiation [2], while static stretch promotes lipid accumulation [3]. Existing research in this field is summarised in Table 1.

Table 1 - Previous research in this field

Type of force	Tension	Compression
Static	✓	✓
Cyclic	✓	✗

- 3D cultures are mostly housed in collagen hydrogels. This results in the inner most cells receiving less nutrients due to diffusion being the limiting factor. To maintain cell viability in long term experiments, a perfusion system is needed.

Aims

- Design a perfusion bioreactor capable of providing mechanical stimulation to a 3D cell culture, to investigate the effects of cyclic compression on adipocytes.
- The end-product must be able to:

1. Allow for simultaneous testing of different strain levels, ranging from 0% to 12%
2. Provide cyclic compression with physiologically relevant frequencies (0.1Hz to 1Hz) [4]
3. Supply uniform delivery of nutrients within the cell-cultured matrix

Acknowledgements

Many thanks to Anabela Cepa Areias, Foivos Chatzidimitriou, Dr. Ester Reina Torres and Kostadin Rolev for their help culturing adipocytes and their insight regarding cellular biomechanics. We would also like to thank Dr. Joseph Sherwood, Mr. Paschal Egan and Mr. Niraj Kanabar for their contribution in the design of the electrical aspects of our device, Mrs. Ji Young Yoon for her help in manufacturing and Dr. Spyros Masouros for helping us improve our computational model of the hydrogel. Finally, we are grateful for the guidance of our supervisor Dr. Darryl Overby who helped us every step of the way.

References

[1] Yuan Y, Gao, J, Ogawa R., Mechanobiology and Mechanotherapy of Adipose Tissue – Effect of Mechanical Force on Fat Tissue Engineering. Plastic and Reconstructive Surgery Global Open. 2015;3(12):e578.
[2] Tanabe Y, Koga M, Saito M, Matsunaga Y, Nakayama K. Inhibition of adipocyte differentiation by mechanical stretching through ERK-mediated downregulation of PPARγ2. Journal of cell science. 2004 Jul 15;117(16):3605-14.
[3] Shoham N, Gottlieb R, Sharabani-Yosef O, et al. Static mechanical stretching accelerates lipid production in 3T3-L1 adipocytes by activating the MEK signaling pathway. Am J Physiol Cell Physiol. 2012;302:C429–C441
[4] Subramanian G, Elsaadany M, Bialorucki C, Yildirim-Ayan E. Creating homogenous strain distribution within 3D cell-encapsulated constructs using a simple and cost-effective uniaxial tensile bioreactor: Design and validation study. Biotechnology and bioengineering. 2017 Aug;114(8):1878-87
[5] Grodzinsky A. Field, Forces and Flows in Biological Systems. Garland Science; 2011

Methods & Design

- The device consists of a pump, driving cell culture medium through 16 bioreactor wells compressed cyclically (Fig. 1).

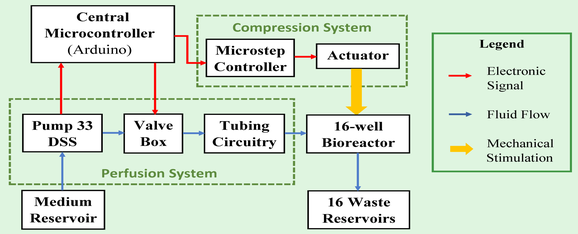

Fig. 1 - Diagram of the complete system

- The bioreactor is comprised of 4 identical groups of 4 wells. Within each group, wells are compressed by strain cylinders of varying lengths, resulting in strains of 0%, 4%, 8%, and 12% (Fig. 2).

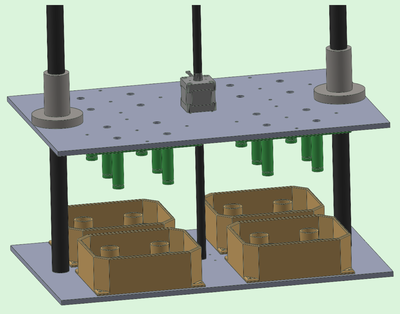

Fig. 2 - Uncompressed state bioreactor

- Sectional view of a single well is shown in Fig. 3. The hydrogel containing cells is placed between the membranes.

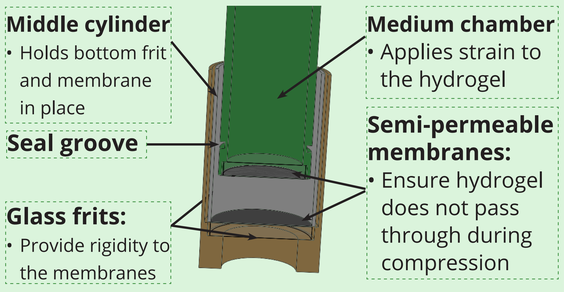

Fig. 3 - One well design

- A pump working in reciprocating mode allows continuous flow through the wells (Fig. 4).

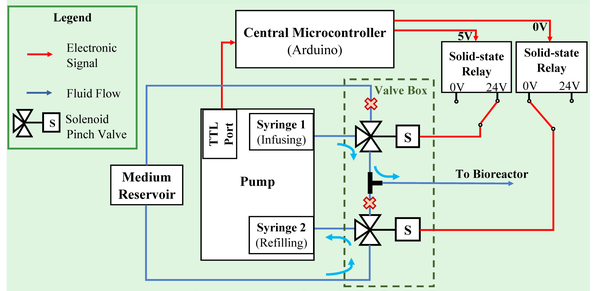

Fig. 4 - Working principle of perfusion system

- A linear actuator provides mechanical stimulation. Micro-stepping is used to improve displacement resolution (Fig. 5). Experimental results are shown in Fig. 8.

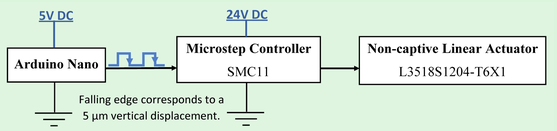

Fig. 5 - Digital control of compression system

- Tendons were extracted from rat tails and solubilized in acetic acid. Four different collagen solutions were created (1, 2, 4 and 8 mg/ml). A sample is shown in Fig. 6.

Fig. 6 - 4 mg/ml Hydrogel

- A cone and plate rheometer was used to determine the viscoelastic properties of the collagen solutions (Fig. 9). The one exhibiting optimal mechanical characteristics was used for the hydrogel scaffold.
- A Finite Element model (in FEBio) was validated by comparison with an analytical solution (Fig. 7). The problem considered was the confined compression of a viscoelastic material using a porous platen [5].

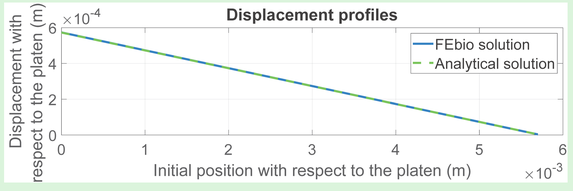

Fig. 7 - Displacement profile of analytical and FEBio solutions. Profiles coincide, hence validating the model

- The model was then modified by making the bottom porous to represent the hydrogel compression. FEBio simulations were performed to determine the volumes whose relaxation times allow cyclical compressions of 0.1 to 1Hz (Fig. 10).

Results

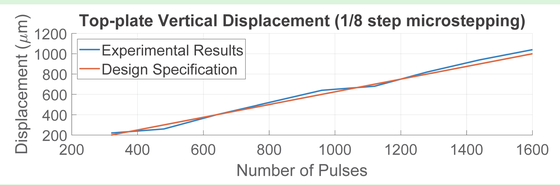

Fig. 8 - Compression plate displacement vs. digital input

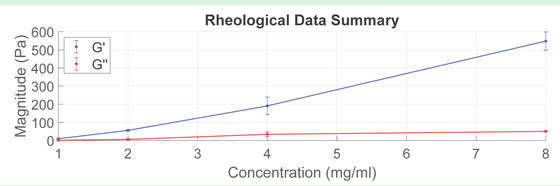

Fig. 9 - Rheometry results.
Storage modulus (G') much larger than loss modulus (G")

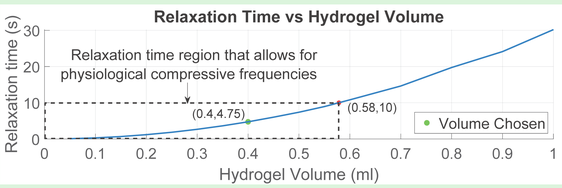

Fig. 10 - Relaxation times for different hydrogel volumes

Further Improvements

- Including a reflective encoder for calibration of compression platform
- Flow sensing for closed loop perfusion control
- Using a separate actuator per strain level to ensure equal compression for all samples
- Adapting a standard multiple well plate instead of 3D printing a custom plate

Conclusion

- A bioreactor prototype that can provide cyclic compression within the physiological frequency range to 3D cell cultures has been successfully designed.
- As the bioreactor can also apply wider strain and frequency ranges, it can be used to study the mechanobiology of various cell lines.
- This device will be utilised to simulate the in vivo conditions of adipocytes, providing more representative results than existing 2D methods.