Investigating the effect of Fluid Flow on Musculoskeletal Cells

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Introduction: Tissue Engineering is a field which aims to find ways to produce new tissue and organs to repair or replace diseased or injured tissues and organs (Tabata, Y, 2009). Part of this process involves cells producing their extracellular matrix to build up their own strength as a tissue (Kelleher, C.M and Vacanti, J.P., 2010) . This project focussed on investigating how fluid flow effects the extracellular matrix (ECM) production of musculoskeletal cells, with the expectation increased fluid flow would increase ECM production.

Methods:

Figure 1

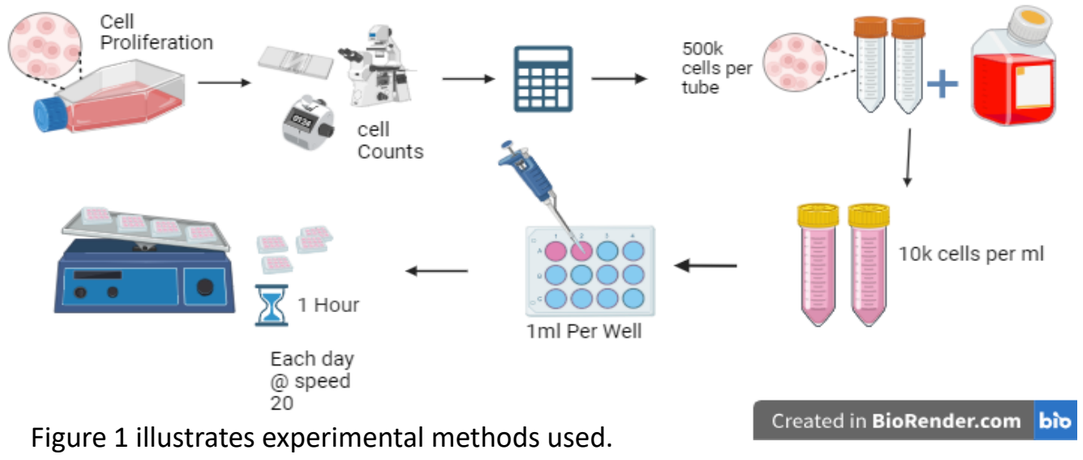

Figure 1 illustrates experimental methods used.

Figure 2

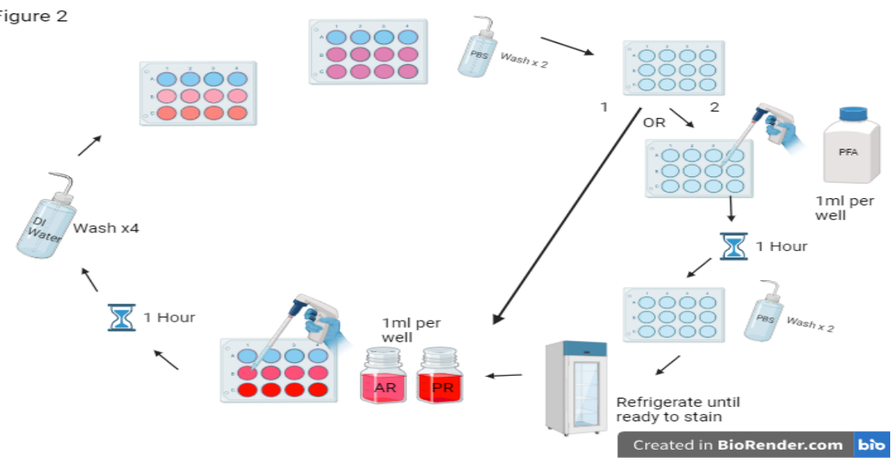

Figure 2 illustrates the staining protocol for alizarin and picrosirius red.

- The experimental set up is shown in figure 1, 8 plates were used for each set up (4 experimental, 4 control) 2 plates were removed from the experiment each week to collect data for 4 time points across the 28 day period. Chicken tendon fibroblast cells (CTF) and differentiated rat osteoblast cells (dRobs) were the cell types used. Cell growth was calculated by doing manual cell counts.
- The Collagen and calcium staining protocol is shown in figure 2 - Cells were stained with alizarin red and picrosirius red, the absorbance of each well was taken to quantify the production of collagen or calcium, these were used as indicators of Extracellular matrix production – due to an error when setting up the experiment calcium production could not be quantified this would be an area for improvement for future studies.

Results:

As shown in figure 4 dRobs cells showed an average increase of collagen production over time, dynamic plates were shown to have lower collagen production than static plates This was an unexpected result, one reason for this be that by day 21 dynamic plates had started showing cell detachment, this would result in a lower number of cells in each well reducing overall collagen production. Within 14 days of starting the CTF experiments all cells on both dynamic and static CTF plates had completely detached and formed a clump this meant accurate data could not be collected for this experiment, and it was decided to adapt the original plan and to compare cell growth of dRobs and CTF cells within the first 7 days it was expected that CTF cells would be much faster proliferating than dRobs. This was not shown by this experiment as seen in figure 6 /7 where dRobs proliferates much faster. Further studies would need to be done to establish what caused the detachment and clumping of the cells.

Figure 3

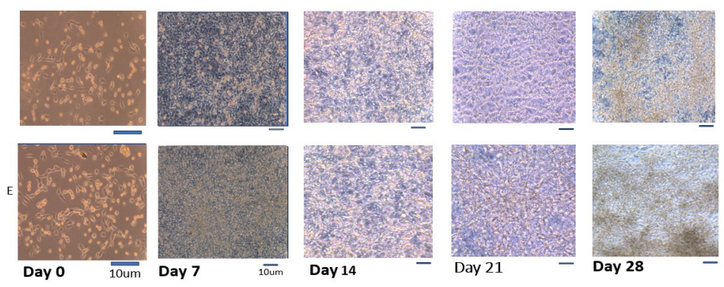

Figure 3: dRobs growth at 5 time point across 28 day period. Experimental/dynamic plates are row labelled E. No notable difference can be seen between control and experimental plates.

Figure 4

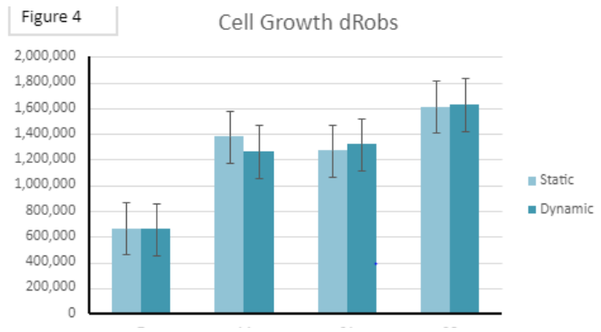

Figure 5

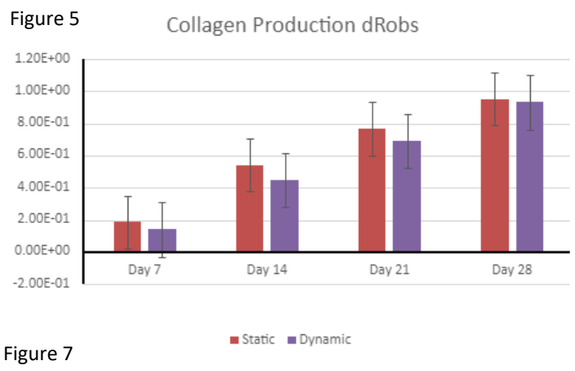

Figure 4 : cell growth of dRobs over 28 day period control showed greater average increase

Figure 5: Collage production quantified by absorbance from staining, control shows on average greater collagen increase over 28 day period

Figure 6

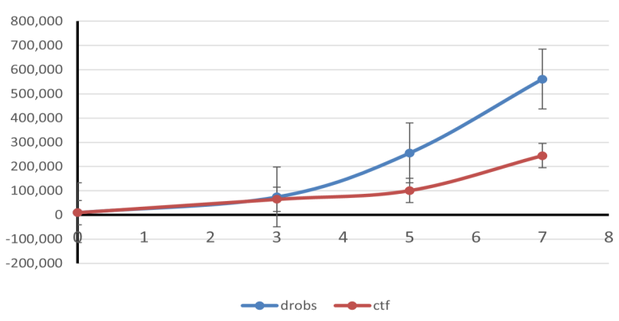

Figure 7

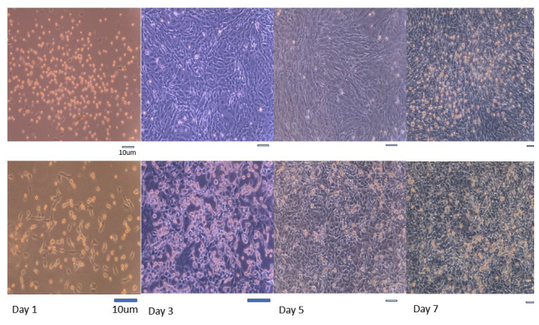

Figure 6: cell growth comparison of dRobs and CTF in first 7 days, dRobs proliferated faster than CTFF

Figure 7: images from 4 time periods across first 7 days of growth illustrating dRobs increased proliferation

Learning and development focus:

⇒ Technical Lab skills i.e. basic cell culture techniques

⇒ Following and adapting protocols when necessary

⇒ Working in sterile conditions

⇒ Learning to work with lab equipment e.g. microscope

I valued the time my supervisor and the PhD students spent with me to develop these skills and gain the knowledge which enabled me to successfully carry out my project.

Next steps for me would be to further my lab training and learn new imaging techniques, I had been keen to try a specific imaging technique during my project but due to the type of plate I had used this was not possible, I would look to resolve this for future work I do.

Knowledge Gained in this subject area: Components of Extracellular matrix – collagen and calcium, How to test for these components – Alizarin red used for calcium staining and Picrosirius red used for collagen staining, basic cell culture techniques.

Project Goals:

✵Gain technical lab skills and experience

✵Develop research skills; experimental design, problem solving and trouble-shooting,

✵Gain confidence in my work and skills

I believe all these goals were met during the project and this is demonstrated by successfully planning, designing, executing and presenting my work.

Other Benefits: I got an insight into what the future could hold for me if I was to pursue a PhD, this is something I am very much looking forward to now based on the positive experience of this project and will continue to work towards this goal using the skills I gained for the remainder of my undergraduate degree.

References:

Kelleher, C.M. and Vacanti, J.P., 2010. Engineering extracellular matrix through nanotechnology. *Journal of the Royal Society Interface*, *7*(suppl_6), pp.S717-S729.

Tabata, Y., 2009. Biomaterial technology for tissue engineering applications. *Journal of the Royal Society interface*, *6(suppl_3)*, *pp.S311-S324.*