Investigating the effect of ultrasound stimulation on human mesenchymal stem cell focal adhesions

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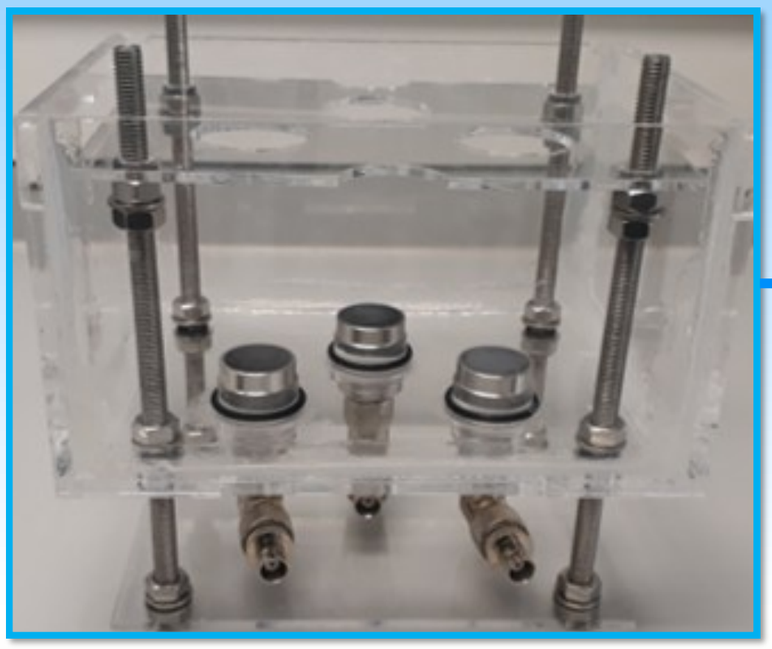
Background and aims

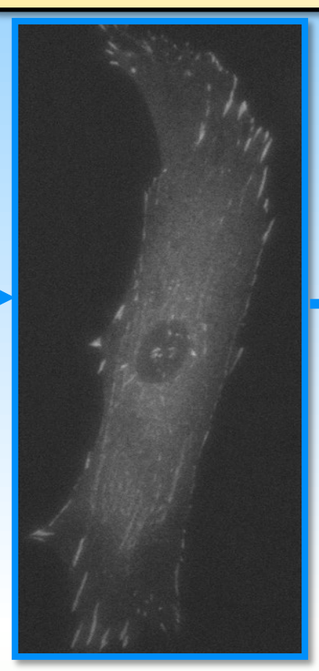
Transmembrane proteins, known as integrins, are used to sense mechanical changes and forces in the local extracellular matrix. These integrins clump together into protein complexes known as focal adhesions. Focal adhesions are important for modulating cellular behaviour and activating mechanotransductive signalling pathways. This project aimed to investigate how low-intensity ultrasound may influence focal adhesion morphology and spatial organisation in human mesenchymal stem cells (hMSCs)

Methods

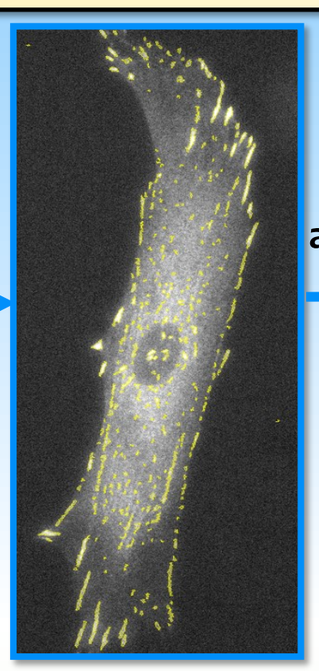
Bone marrow derived human mesenchymal stem cells (BM-hMSCs) were stimulated by three ultrasound transducers submerged in water, operating at 1.11 MHz with 2.5W. Stimulation durations were 0, 20, 60, and 180 minutes, once per day over a course of three days. Cells were fixed and immunostained for vinculin to visualise focal adhesions. Fluorescence microscopy was performed with a 20x objective with consistent imaging settings across all samples to allow for comparisons between focal adhesion morphology. These images were then imported into the focal adhesion analysis server (FAAS) to acquire quantitative data on focal adhesion area, adhesion signal intensity, orientation, major and minor axis length, eccentricity, orientation, and spatial properties relative to the cell centroid. Focal adhesion properties were analysed in GraphPad Prism V10.6.1 to examine variability, differences between stimulation times, and property correlations.



Cell imaging



FAAS

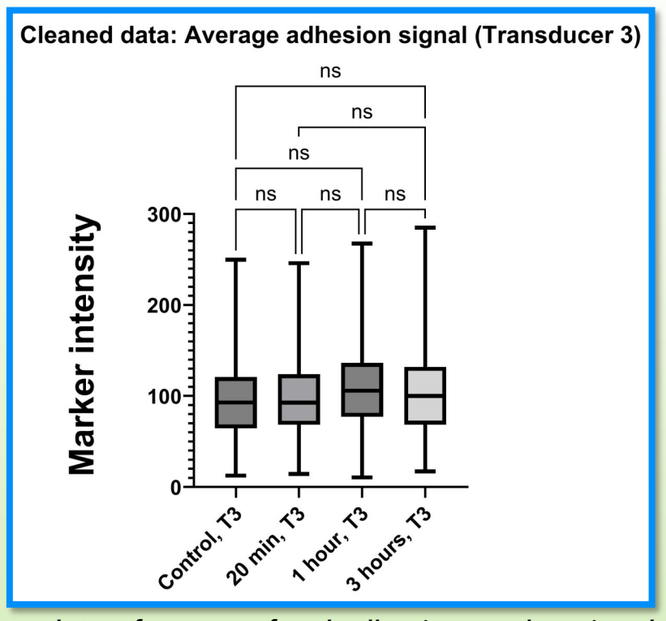


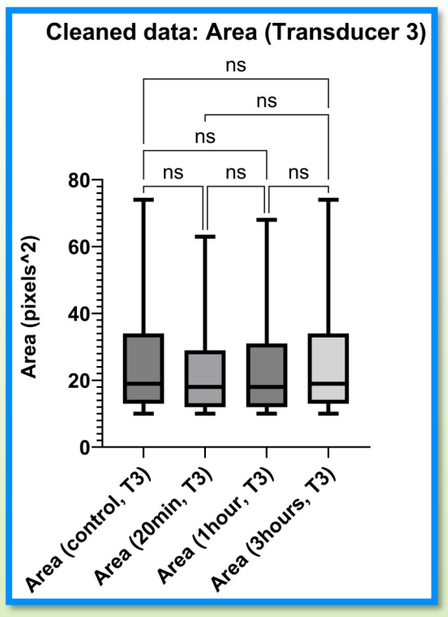
Data acquisition

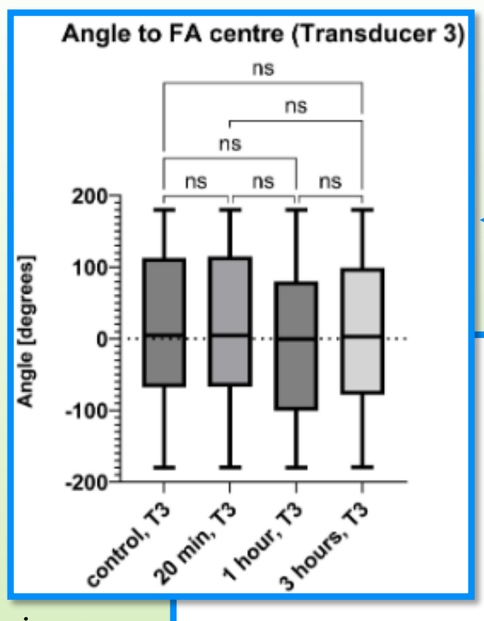
	Group A	Group B	Group C
	Area (control, T1)	Area (20min, T1)	Area (1hour, T1)
1	59.00	142.00	21.00
2	20.00	14.00	17.00
3	140.00	17.00	15.00
4	78.00	121.00	10.00
5	32.00	28.00	40.00
6	155.00	112.00	27.00
7	695.00	45.00	97.00
8	10.00	64.00	57.00
9	15.00	104.00	26.00
10	23.00	65.00	44.00
11	10.00	156.00	19.00
12	97.00	22.00	25.00
13	38.00	11.00	124.00
14	107.00	64.00	32.00
15	13.00	75.00	54.00
16	21.00	170.00	78.00
17	10.00	112.00	13.00
18	68.00	31.00	39.00
19	17.00	47.00	13.00
20	33.00	11.00	10.00
21	137.00	12.00	32.00
22	12.00	49.00	22.00

Statistical analysis

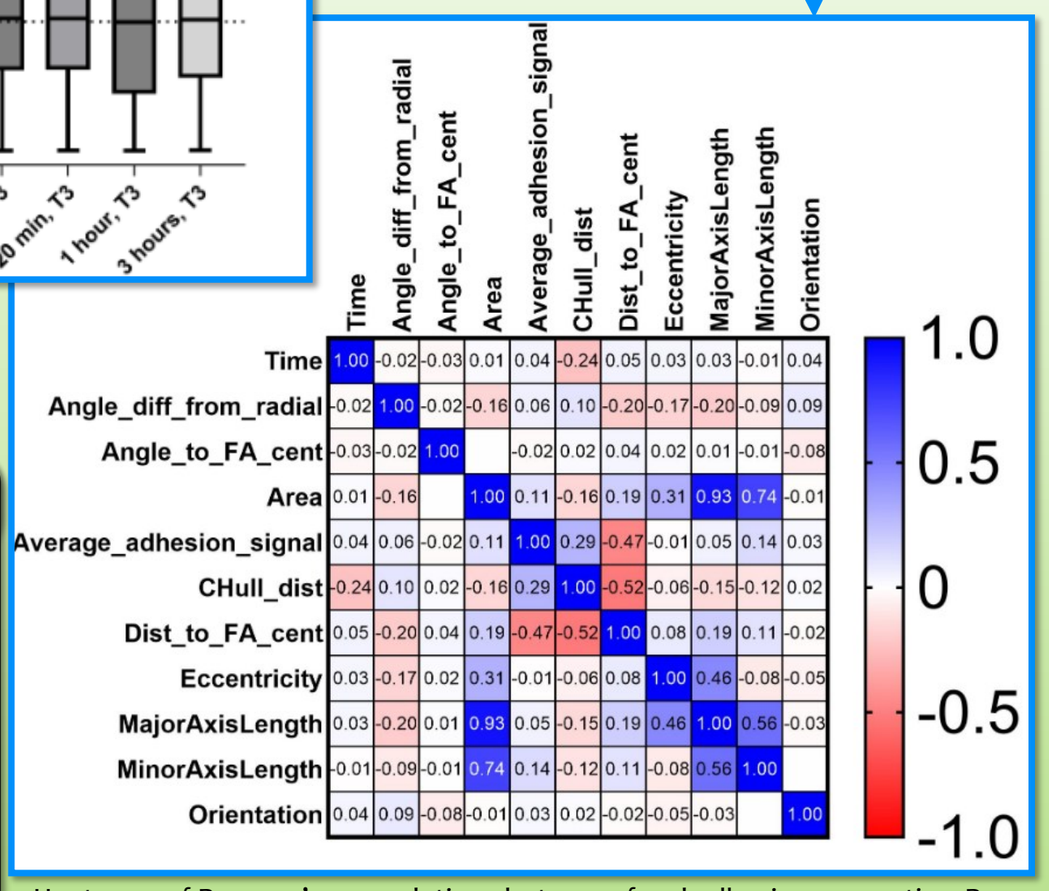
Prism







Box plots of average focal adhesion marker signal, focal adhesion area, and angle to focal adhesion centre. Outliers removed with the ROUT method (Q=1%). Data represents mean with 25th and 75th quartiles, with minimum and maximum values as whiskers. ns, not-significant



Heat map of Pearson's r correlations between focal adhesion properties. Property data was acquired from the Focal Adhesion Analysis server from human mesenchymal stem cell stimulated with ultrasound for 0, 20, 60, and 180 minutes

Results

Despite variability of results across various stimulation times, no statistically significant differences were observed. This may be due to biological variations, analytical sensitivity, or other responses that are currently not explored within this current experimental setup. Limitations such as imaging resolution, inhomogeneous stimulation gradient, sample sizing, and focal adhesion segmentation may have limited accurate characterisation of focal adhesions. Future experiments that improve on these limitations, whilst also employing the usage of further mechanobiological markers, could provide greater insight into ultrasound-mediated cellular responses.

Conclusion

The importance of focal adhesions in mechanotransduction has been reported frequently in literature. Whilst no definitive or consistent trends were observed with these experimental parameters, these results provide an initial characterisation of focal adhesion behaviour. With improvements to methodologies and expansion of experimental parameters, future experiments will be able to achieve a greater understanding of the relationship between ultrasound stimulation and focal adhesion dynamics in human mesenchymal stem cells.

References

- Berginski ME, Gomez SM. (2013) The Focal Adhesion Analysis Server: a web tool for analyzing focal adhesion dynamics [v1; ref status: indexed, http://f1000r.es/yc] F1000Research 2013, 2:68 (doi: 10.3410/f1000research.2-68.v1)