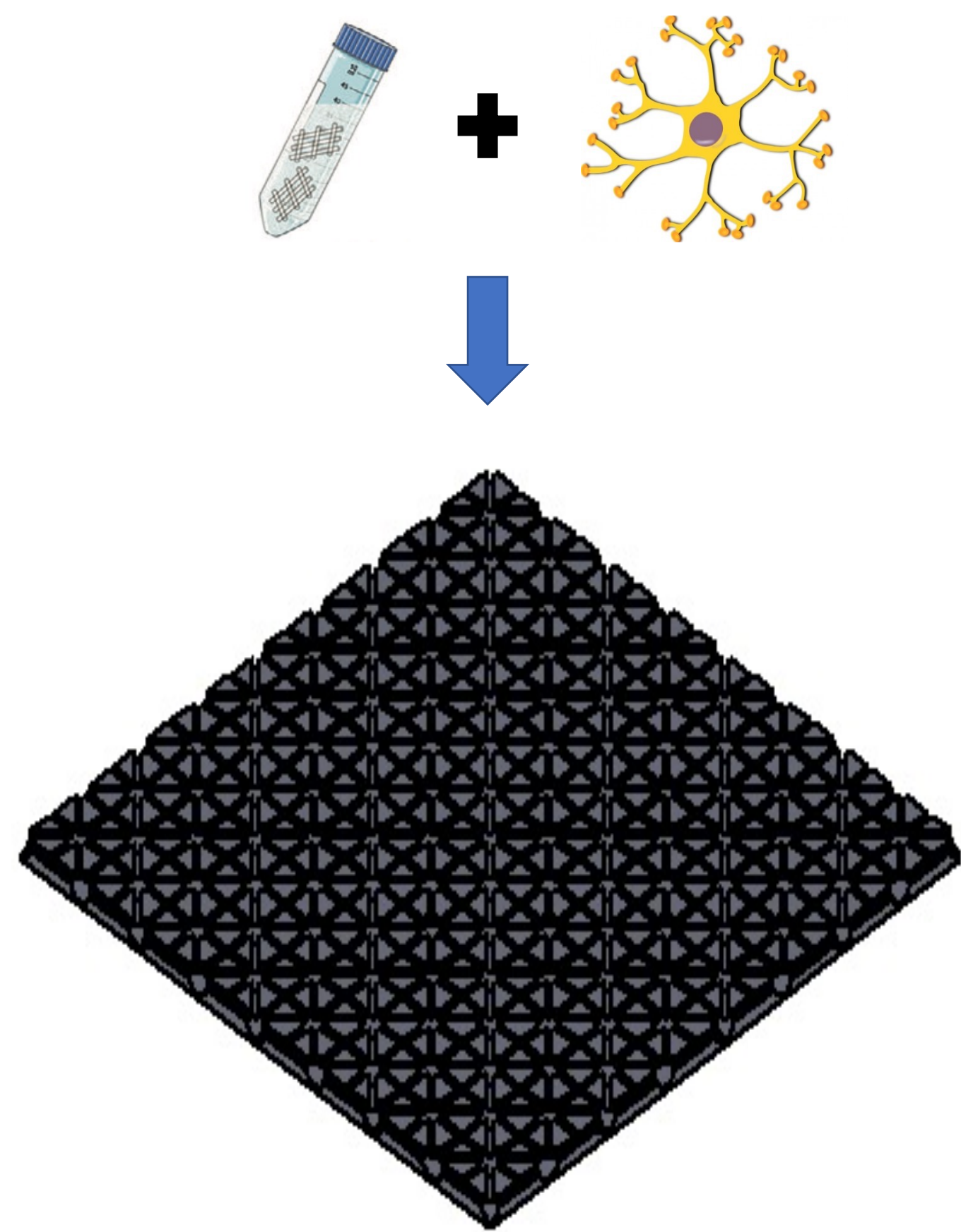


Development of a physiologically relevant osteocyte network in vitro to model bone pathophysiology

Rucha Benare, Dr David Hoey

School of Mechanical, Manufacturing & Biomedical Engineering, Trinity College Dublin



Problem

Osteoporosis is a fatal disease that has affected more than 200 million patients. Current research to test drugs for their treatment is majorly done in vivo which unfairly relies on animal testing without improved efficacy for the gains of utilizing such strategy. Thus, there exists a significant demand to make more human relevant models on which the effectiveness of a variety of drugs could be tested.

Solution

One such attempt is using Direct Laser Writing, an advanced 3D printing technology, to design a structure that acts as a scaffold on which the osteocyte network could be grown. The research aimed to develop this micro-physiological model by creating an idealized chip called 'osteochip' made of mainly Trimethylolpropane ethoxylate triacrylate using computer-aided design (CAD) software called SOLIDWORKS.

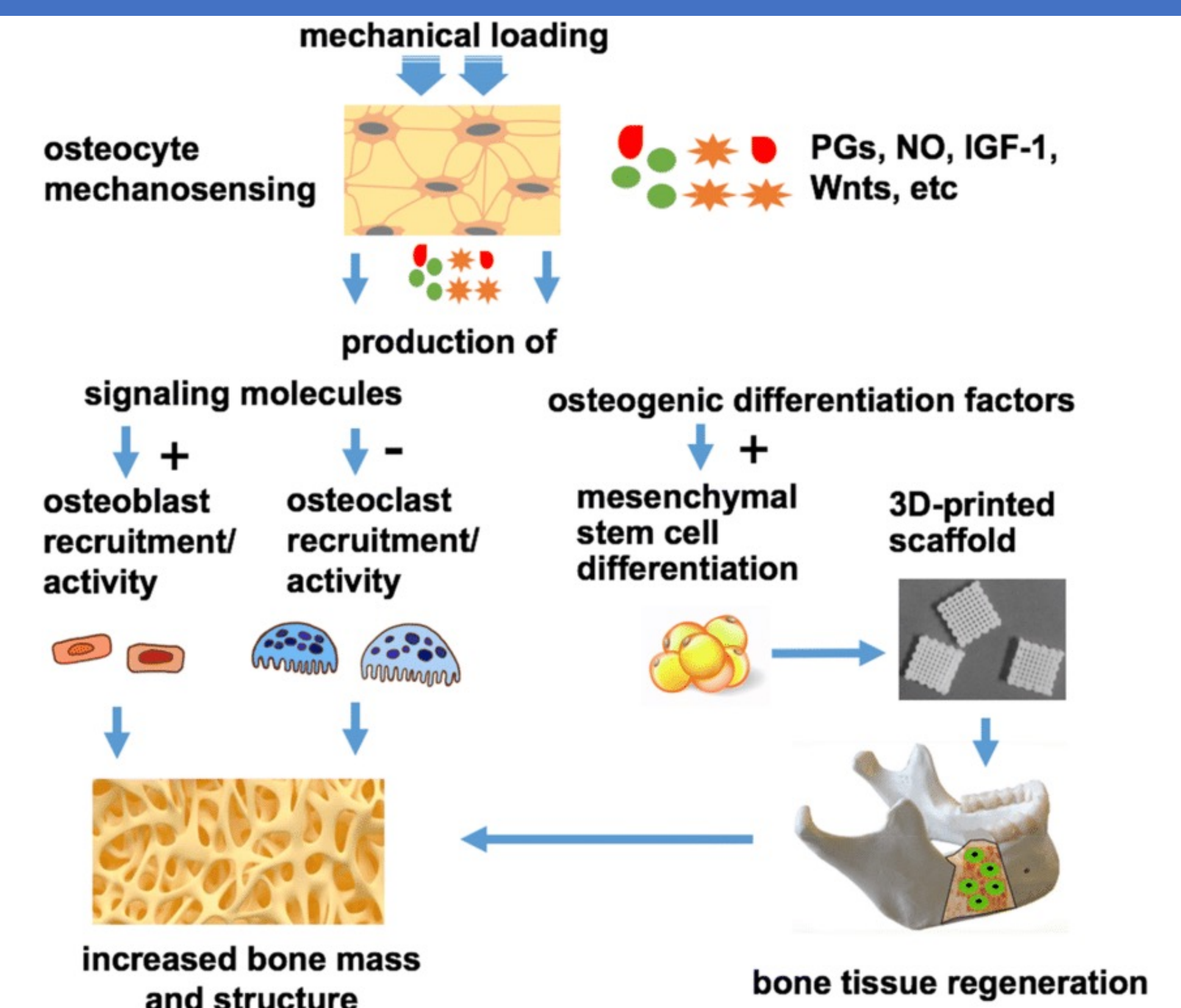


Figure 1 Overview of various elements at play during mechanotransduction and the role of osteocytes in bone tissue

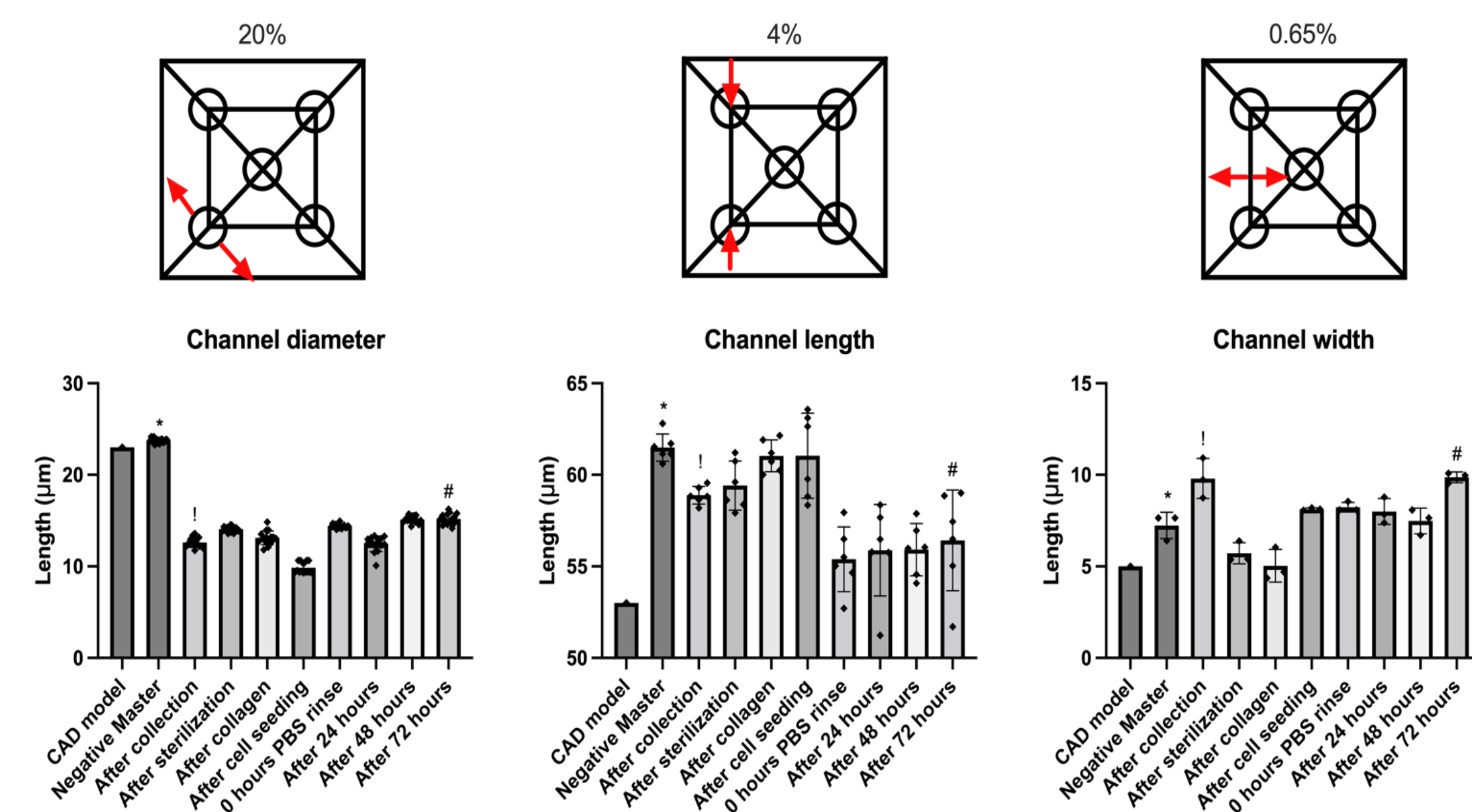


Figure 2 Characterisation of the changes in the channels via measuring diameter, length, and width randomly across the span of the chip. The measurements are again taken at different stages of processing of the chip; as a CAD model rendering, after 3D printed as Negative master, after collection of the idealised version, and so on. All statistical significances (* ! #) are compared to measurements of CAD model via ordinary one-way ANOVA and Tukey post-test. * = $p < 0.0001$, ! = $p < 0.0001$, # = $p < 0.0001$.

Osteochip, an Idealized Chip

Bone is a living tissue that is self-constructing and perpetually repairing to maintain its homeostasis. At the microscopic level, the intricate 3D nature of the bone microenvironment encompasses a complex architecture that houses innervation, vasculature, and different types of bone cells, with osteocytes being the abundant ones. Figure 1 provides an overview of some recent research in understanding the role of osteocytes in regeneration of bone tissue [3]. Thus, an idealized osteochip was designed using SOLIDWORKS to attempt this architecture. It was sterilized, coated with collagen and nano-needle Hydroxyapatite (nnHA) coating and analysed for the chip shrinkage over time as seen in figures 2 and 3. The shrinkage was attributed to effects of polymerization that is common to methacrylates [4]. The construct was then seeded with murine osteocyte-like cell line called MLO-Y4 and stained using DAPI/Actin to observe the development of osteocytes as shown in figure 4. The results called for an improved method of preferential coating of the chip. This could be done by making the wells hydrophilic to attract and house bone biomimetic environment while maintaining the planar surface of the chip hydrophobic -in order to further direct the coatings into the wells.

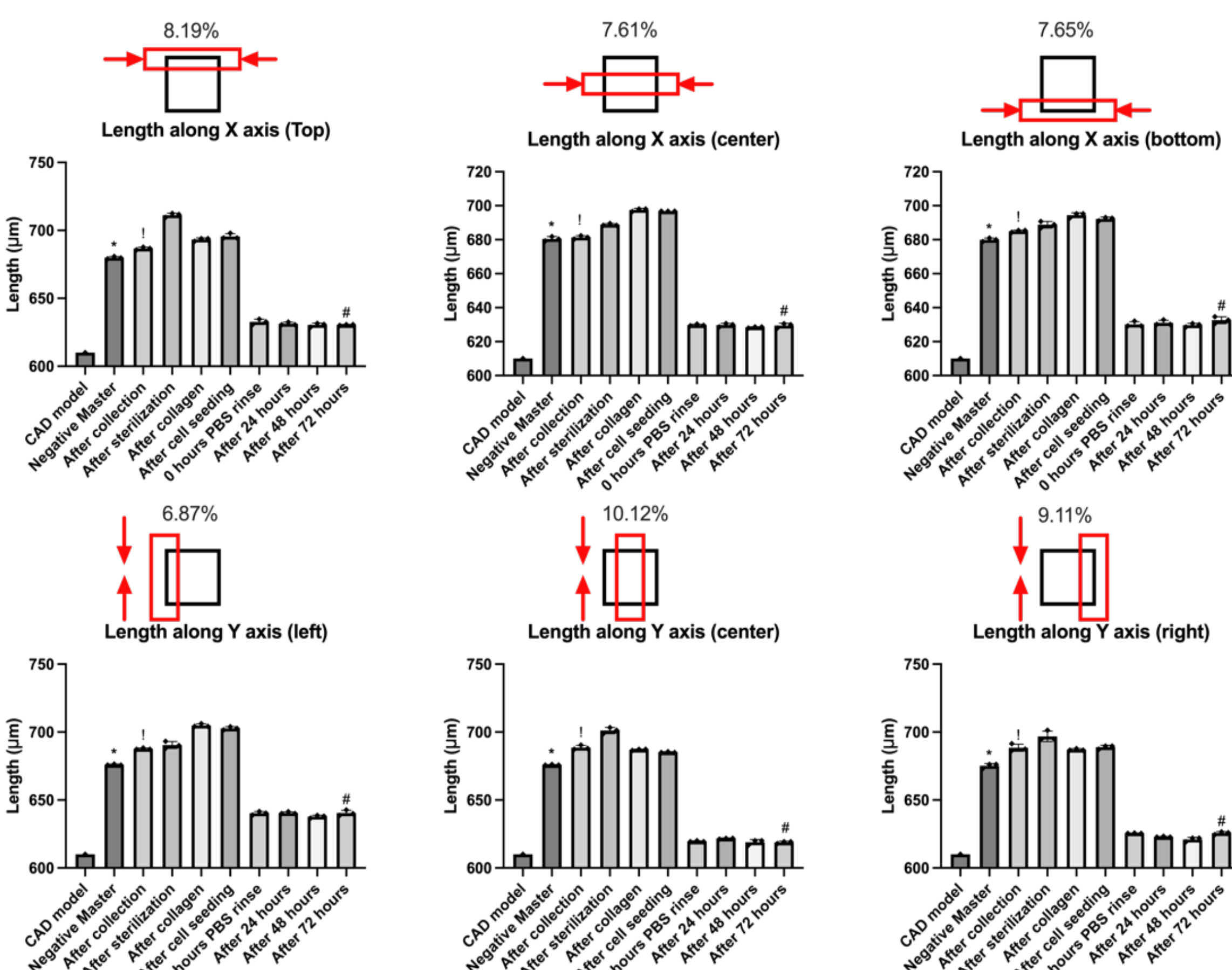


Figure 3 Characterisation of the shrinkage of chip via measuring lengths of side of the chip. The red highlights the side of the chip (black) under observation. The measurements are taken at different stages of processing of the chip; as a CAD model rendering, after 3D printed as Negative master, after collection of the idealised version, and so on. All statistical significances (* ! #) are compared to measurements of CAD model via ordinary one-way ANOVA and Tukey post-test. * = $p < 0.0001$, ! = $p < 0.0001$, # = $p < 0.0001$.

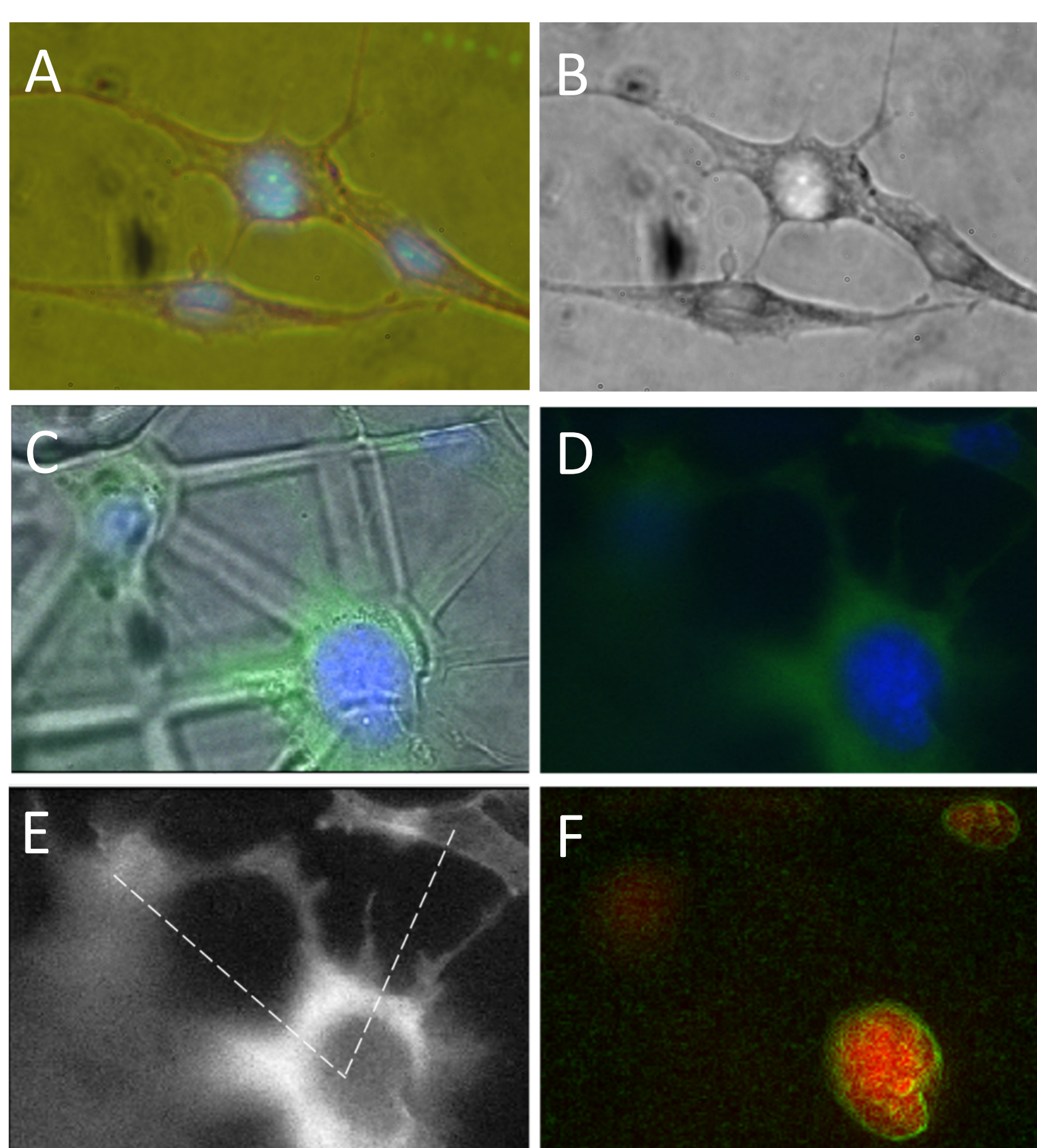


Figure 4 Development of osteocytes without the idealised chip (A,B) and using the idealised chip (C,D,E,F)

A) And B); Brightfield imaging of osteocytes growing 31 hours after cell seeding. Images further processed via ImageJ Z stack projection for A) spectrum colour code B) SUM of all the slices present.

C) and D); Cells on the osteochip with DAPI Actin staining observed under fluorescence imaging using IX83 at 100x magnification. Images processed with CellProfiler, open source imaging software

E) Features 'ColorToGray' and 'EnhancedEdges' to distinguish the branching out of the processes. The angles between the processes developing from the same cell are observed to be congruent with the angles between the channels of the chip on which they have been positioned.

F) Composite image of the nuclei highlighted from the same image for further clarity on the positioning of the cells with relation to the wells of the chip

Conclusion

In summary, direct laser writing proved to be a very useful tool in creating an idealized micro-physiological model to make a human-relevant model. The model, an idealized osteochip, was observed to have an overall planar shrinkage. The channels had minor and wells themselves had significant expansions over time. This consecutively called for more refinement in the area of updating the dimensions, the ratio of the coatings used, the material that has been used to print, and techniques for further improving the coating strategy. One of possible techniques would be to devise a combination of hydrophobic planar surface of the chip and hydrophilic cavity structure to induce preferential coating. Overall, the notion of the idealized chip as a micro-physiology model holds exciting promise in truly understanding how mechanical stimuli transduce cells into their subsequent cascade of intracellular mechanisms which in turn affect and remodel the bone, and aid in curing diseases like osteoporosis.

Acknowledgements

I would sincerely like to thank Dr David Hoey and his team for helping and guiding me initiate research in such a brilliant and exciting field of science. I would also like to thank Laidlaw foundation for providing me with the opportunity to truly appreciate and undertake a part in ethical scientific research.

References

- Sözen, T., L. Özışık, and N. Başaran, An overview and management of osteoporosis. Eur J Rheumatol, 2017. 4(1): p. 46-56.
- Cao, W., et al., Is There a Governing Role of Osteocytes in Bone Tissue Regeneration? Current Osteoporosis Reports, 2020. 18.
- El-Fiqi, A., J.H. Kim, and H.W. Kim, Novel bone-mimetic nanohydroxyapatite/collagen porous scaffolds biomimetically mineralized from surface silanized mesoporous nanobioglass/collagen hybrid scaffold: Physicochemical, mechanical and in vivo evaluations. Mater Sci Eng C Mater Biol Appl, 2020. 110: p. 110660.
- Schricker, S.R., 9 - Composite resin polymerization and relevant parameters, in Orthodontic Applications of Biomaterials, T. Eliades and W.A. Brantley, Editors. 2017, Woodhead Publishing. p. 153-170.