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Real-time Visualisation of Actin Filaments within Native and Tissue Engineered Cartilage using SiR-Actin

C. K. Kwan[1], S. Finlay[2], D. Wood[2], B. B. Seedhom[2], J. Kirkham[2];

[1] Department of Oral Biology, Institute of Medical and Biological Engineering, Leeds, UNITED KINGDOM.

[2] Department of Oral Biology, School of Dentistry, Leeds, UNITED KINGDOM.

Introduction: We recently reported the use of incremental compressive loading to generate cell-scaffold constructs with high modulus and cartilage-like histology.[1] Our overall aim is to improve the efficacy of construct growth, accelerating clinical translation. We asked whether a specific range of cellular deformation promotes optimal deposition of "quality" cartilage-like ECM in maturing constructs. Our aim was to develop a staining method for real-time visualisation of cellular deformation of living cells within constructs.

Method: Three scenarios were used for experimentation; i) Bovine synoviocytes cultured in well plates for 7 days, ii) cartilage plugs obtained from bovine knee joints and iii) tissue engineered constructs generated by dynamically seeding bovine synoviocytes on non-woven polyethylene terephthalate fibre scaffolds and cultured in chondrogenic medium for 12 weeks. Actin filaments were stained with recently developed SiR-actin[2] (0.1 μM) for 12h at 37°C and nuclei stained with Acridine orange (1 μg/mL) for 30 min at 37°C in DMEM/F12 medium.

Results: High magnification confocal images of cell monolayers showed specific and highly resolved staining of cytoskeletal elements and cell nuclei. 3D images of native cartilage and tissue constructs showed similar staining.

Conclusion: A staining methodology was optimised using a new staining molecule that can penetrate 3D constructs and native cartilage with good resolution. Cells remained viable thoughout. The method is suitable for real-time experiments to determine cell deformation under compression to optimise construct maturation in the longer term.

References

1. Finlay et al. Tissue Eng Part C. 22. 2016.
2. Lukinavičius et al. Nature Methods 11:7. 2014.