Deciphering the Roles of Ageing & Mechanotransduction in Rotator Cuff Tendon Healing

Asawari Parulekar [1], Aswathi Gopalakrishnan[1,2], Eleonore Bolle[1], Lisbeth Grondahl [3,4,], Justin Cooper-White[1,4,*]*

[1] School of Chemical Engineering, The University of Queensland, Australia.
[2] School of Biomedical Sciences, The University of Queensland, Australia.
[3] School of Chemistry and Molecular Biosciences, The University of Queensland, Australia.
[4] Australian Institute for Bioengineering and Nanotechnology (AIBN), The University of Queensland, Australia.

* l.grondahl@uq.edu.au, j.cooperwhite@uq.edu.au

With a globally ageing, but more active population, musculoskeletal tissue injuries are of increasing prevalence in our society. Rotator Cuff (RC) tears, resulting in severe pain and instability in the shoulder, are one such age-related injury, with increasing incidence with age (e.g. 62% of the population above 80 years are affected). Retear rates post-surgery also compound with patient age and can be as high as 94%. Ineffective, fibrotic repair at the tendon-to-bone attachment is the most prominent cause for retears. It has been hypothesised that such poor healing is due to age-related alterations in mechanotransduction (*mechanoageing*) of reparative cells in this tissue. However, the impacts of mechanoageing of resident perivascular stem cells (or pericytes) in the vascular sheath surrounding the tendon (often referred to as perivascular tendon stem/progenitor cells (TSPCs)), known to contribute to tenogenesis and repair, remains unclear. This lack of clarity is due to, in most part, the difficulty in obtaining healthy human RC tissues from young through to old cohorts, and the significant genetic and epigenetic variations across donors. This study aimed to develop a human induced pluripotent stem cell (hiPSC)-based model for cellular ageing to elucidate the contributions of pericyte (or TSPC) mechanoageing to fibrotic tendon repair. A hiPSC line with a doxycycline (DOX)-inducible ageing cassette driving progerin overexpression (reported in real-time by an eGFP-progerin fusion construct) was used to create an *in vitro* ageing model for mesenchymal lineage tissues. hiPSCs were differentiated into hiPSC-derived pericytes. We first confirmed that these pericytes were analogous to perivascular tendon stem/progenitor cells (TSPCs), making this an ideal platform to study the impacts of ageing on tendon regeneration. Thereafter, time-dependent effects of DOX-induction on hiPSC-pericytes was quantified over a period of 14 days using immunofluorescence, flow cytometry, rt-qPCR and SA-β-gal. In the first 7 days post-induction, hiPSC-pericytes exhibited key hallmarks of ageing, including dysmorphic nuclear membranes and altered expression of nuclear mechanoregulatory proteins (Lamin B1, LAP2α), typical biomarkers associated with age-related cell dysfunctionalities, especially senescence, and loss of mechanosensing capability. However, by 14 days following DOX-induction, hiPSC-pericytes entered a pro-inflammatory state and demonstrated a complete reversal in phenotype, lacking typical progeria-induced signatures such as enhanced matrix deposition, cell cycle arrest and senescence. These results indicate aged pericytes/TSPCs 1.) uniquely possess protective mechanisms that provide resilience over time against typical downstream impacts of age-related progerin expression; 2.) do not differentially contribute to age-related fibrosis-dominated repair; and 3.) instead contribute to failed tendon repair after injury in the aged by entering a chronic pro-inflammatory state during the early stages of wound healing, affecting their tenogenic potential. These insights have significant impacts on tissue engineering strategies that rely on TSPCs for regeneration of rotator cuff tissues – our work on multimodal scaffolds to address these challenges will be detailed and discussed.