MULTIMODAL LABEL-FREE IMAGING FOR QUANTITATIVE ASSESSMENT OF BIOENGINEERED TISSUE

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KEY WORDS: Non-linear microscopy, multimodal imaging, live imaging, 3D imaging, skeletal stem cells

Musculoskeletal disorders are the second most common cause of disability worldwide with diseases such as osteoarthritis (OA) expected to continue to rise with an increasingly obese, sedentary and ageing population [1]. Therefore, understandably, research is focused on the development of reparative & restorative treatment strategies with skeletal stem cells showing significant promise. However, key to translation of stem cell based strategies is an enhanced understanding of their *in vivo* integration and objective assessment of the treatment competency of the *in vitro* engineered live tissue in a label-free, non-invasive and non-destructive manner.

In this talk I will describe our work to gain a quantitative understanding and to objectively assess cell behavior and tissue development for skeletal repair and regeneration using multimodal non-linear techniques such as coherent anti-Stokes Raman scattering (CARS), second harmonic generation (SHG) and two-photon excited autofluorescence (TPEF). These techniques interrogate the chemical and structural composition allowing us to study the differentiation of skeletal stem cells (SSCs) and visualisation of temporal changes accompanying adipogenesis [2] and chondrogenesis [3] in a completely label-free, non-invasive and non-destructive way. We have also verified both visually as well as through gene expression analysis that SSC differentiated live tissue constructs remain viable and are unaffected by CARS and SHG imaging. Furthermore, elucidation of the architecture of the differentiated tissue is especially important for cartilage tissue engineering. The application of 3D in place of 2D imaging has enabled us to obtain a comprehensive understanding of the collagen fibre network during the chondrogenic development of SSCs. Quantitation of the various molecular and structural readouts allowed us to develop assessment parameters to track the differentiation of SSCs and their *in vitro* tissue development. The non-invasive and non-destructive 3D imaging opens new avenues for real-time applications while the label-free quantitation provides unprecedented insight and characterisation of the development stages of skeletal 'engineered-tissue' in the clinic for optimal use in therapy.

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