NON-LINEAR MULTIMODAL IMAGING FOR SKELETAL STEM CELL CHARACTERISATION

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Bone fractures, among other orthopaedic conditions, constitute a significant clinical and socio-economic problem, exacerbated by an aging population. The use of skeletal stem cells (SSCs) for cell-based therapies is currently one of the most promising areas for skeletal tissue repair and disease treatment [1]. Current limitations in the characterisation of SSCs have led to the application of alternative strategies that aim to identify molecules at the subcellular level by using their inherent properties, without the use of any dye or label, i.e. "label-free" [2]. Label-free methods such as coherent anti-Stokes Raman scattering (CARS) and second harmonic generation (SHG) microscopy are minimally invasive, non-destructive, and are emerging as powerful alternatives to conventional techniques in biomedicine [3]. The non-linear techniques CARS and SHG can be further combined with two-photon (auto)fluorescence imaging to provide complementary molecular information.

In this work, human SSCs were differentiated under *in vitro* pellet culture conditions into chondrocytes (cartilage) over 21 days and examined using label free spectroscopic methods [3]. Raman spectra provided molecular information on the SSCs and the ability to distinguish biochemical changes in skeletal stem cell differentiation. Multimodal imaging (CARS, SHG and autofluorescence) in 3D (Figure 1) revealed for the first time lipid distribution within the cell pellet. By using 3D instead of 2D imaging it was also possible to achieve a more comprehensive understanding of the collagen fibrous network in SSC pellet cultures. The SHG imaging showed increased collagen expression and curvature (fibril pattern) with maturation of the chondrogenic pellet culture. Additionally, 3D SHG imaging allowed to distinguish collagen fibre organisation in different areas of the pellet during the chondrogenic development process of SSCs.

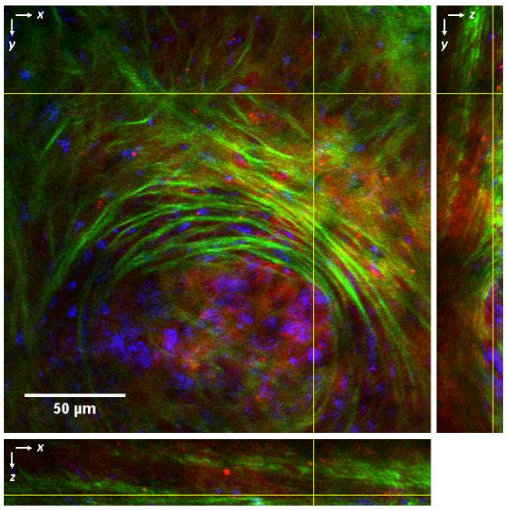

Figure 1 – 3D multimodal imaging of SSC chondrogenic culture (red: CARS, green: SHG, blue: autofluorescence). Orthogonal views (*xy*, *xz* and *yz*) show the intersection planes at the position of the yellow cross-hair.

Overall our studies demonstrate the utility of non-linear multimodal imaging and the potential of label-free approaches and their application to the field of therapeutics and skeletal repair and regeneration research.

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