LIGHT SHEET FLUORESCENCE MICROSCOPY IMAGING FOR THE DEVELOPMENT OF AN *IN VITRO* TISSUE BOUNDARY 3D TUMOUR MODEL USING SELF-ASSEMBLED HYDROGEL

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Multicellular Tumour spheroids (MCTS) have become increasingly popular and widely regarded as an excellent 3D in vitro model to examine tumour drug penetration [1,2] and the influence of tumour microenvironment [3]. Yet, imaging MCTS has been challenging using conventional confocal or multiphoton microscopy, due to their limited depth, working distance and phototoxicity as well as the specimen preparation.
Light sheet fluorescence microscopy (LSFM) overcomes many of these issues [4] and here we describe the specimen preparation of spheroids for 4D imaging via Zeiss Lightsheet Z.1 and the Leica TCS SP8 DLS. We have used these preparations to examine SK-N-BE(2) neuroblastoma spheroids "sandwiched" between two hydrogels (asymmetric model).
Our aim is to mimic the growth of a tumour on the boundaries between two tissue types with extracellular matrix (ECM) of different mechanical properties and morphologies.
Self-assembled peptide hydrogels are a subclass of biomimetic materials able to mimic the nanostructure of the ECM [5] in biomedical applications. They are often easily functionalized, stable in common cell culture media and optically transparent. Moreover, the versatility of these biomimetic materials allows us to display a wide range of physiological-like mechanical properties characterized by Atomic Force Microscopy Nano indentation.
The use of tunable hydrogel scaffolds as ECM mimics for MCTS LFSM 4D imaging provides an adaptable mechanotransduction platform for an in vitro tissue boundaries tumour model.

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