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P297 Towards bone biomimetic in vitro osteocyte models using micro-3D printing

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Introduction: Osteocytes, the most abundant cells in bone, are responsible for sensing the strains that bone is subjected to [1]. In vivo, they reside in a system of cavities and sub-micron channels in the bone matrix termed lacuno-canicular network (LCN), and this three-dimensional (3D) environment strongly influences the mechanical signals that osteocytes perceive [2,3]. However, this important aspect is not captured by conventionally used systems to investigate osteocytic mechanosensing such as parallel plate flow chambers. To overcome these limitations and study the influence of the 3D environment on osteocyte mechanosensing, we aim to establish an in vitro system for functional live cell studies in biomimetic 3D structures. Methods: Structures resembling the LCN were fabricated by micro-3D printing with the Nanoscribe Professional GT, a system based on two photon polymerization, using OrmoComp, an inorganic–organic hybrid polymer, as the printing ink. IDG-SW3 osteocytes [4] were grown on planar, collagen-coated OrmoComp substrates and cell viability was determined by LIVE/DEAD staining. Cells were reseeded on tissue culture plastic following collagenase/trypsin digestion. Results: Networks of channels and cavities were successfully fabricated by micro-3D printing (Fig. 1A). Channel diameters down to 2.5 µm for a channel length of 20 µm could be achieved (Fig. 1B). On OrmoComp, IDG-SW3 cell survival was consistently high (>95%) over 35 days of culture. IDG-SW3 cells reseeded after 35 days exhibited an osteocyte-like dendritic morphology (Fig. 1C). Discussion: Micro-3D printing of OrmoComp was found to be suitable for creating structures resembling the LCN. Furthermore, the material enabled long-term culture of osteocytic IDG-SW3 cells and observation by fluorescence microscopy. Live cell imaging of osteocytes in the printed structures is currently addressed in ongoing studies.

Figure 1: A Micro 3D-printed cavity and channel structure. B Scanning electron micrograph of a focused ion beam cross-section from the center of a 20 µm channel in OrmoComp. C Dendritic morphology of IDG-SW3 cells after reseeding (green: cytoplasm (Calcein AM), blue: nuclei (Hoechst)). Scale bars: A, C: 30 µm, B: 5 µm.