TWO-PHOTON POLYMERIZATION FOR 3D CANCER ORGANOID SCAFFOLD PRINTING

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Recently, 2D cell cultures and laboratory animals have been considered the standard for modelling cancer. While 2D cultures offer simplicity, cost-effectiveness, and well-established methods for studying cancer cell behaviour, they fall short of accurately replicating the intricacies of native tumour tissue. These cultures cannot mimic the biological, chemical, and mechanical cues present in native 3D tissues [1,2]. However, the use of animal systems come with significant costs and notable limitations in terms of controllability, reproducibility, and design flexibility. Moreover, the adoption of the 3R (Replacement, Reduction, and Refinement) principle in animal research discourages the prevalent use of animal systems for primary cancer research. A compelling prospect surpassing both of the discussed methods involves utilizing 3D platforms to bridge the gap between 2D cell cultures and intricate animal models [3]. Conversely, the ongoing development of 3D cell culture systems using cell scaffolds hold promise in replicating the complexity of *in vivo* tumors. These 3D models offer soluble gradients, enabling adhesion distribution in all three spatial dimensions without polarity. The ability to facilitate 3D adhesion, along with the incorporation of relevant biomechanical cues and gradients, positions 3D scaffold-based cancer cell models as promising platforms for testing drug-delivery technologies, exploring pluripotency and self-renewal, initiating primary cultures from patients, studying interactions between tumours and the immune system, and identifying predictive biomarkers for potential use in future clinical cancer treatments [4].

Achieving an optimal cancer cell modelling system is challenging. It requires considerable effort due to the processing techniques available for designing and fabricating 3D tissue models that fully enhance cellular performance *in vitro*. In this study, we focus on cancer tumour organoid formation. We used two-photon polymerization (TPP) for 3D scaffold printing [5]. TPP represents an innovative photolithographic method employing femtosecond laser pulses, facilitating unrestricted 3D micro structuring of liquid photo resins. This is achieved by leveraging the interplay between axial and lateral spatial confinement of the photoreaction within the focal volume of a concentrated laser beam. It allows to create more sophisticated 3D structures in great detail. Therefore, we use this 3D fabrication technology to analyze the formation of cancer organoids on different polymers and scaffolds.

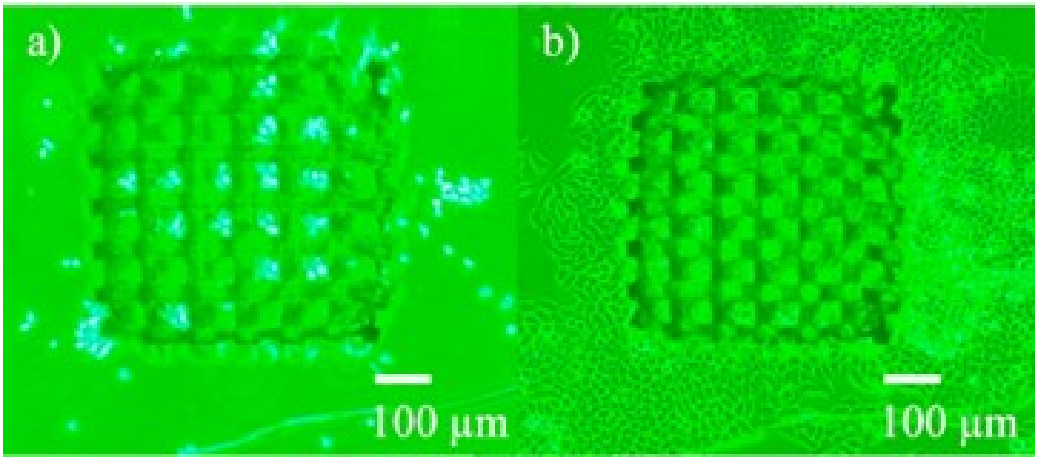

Fig. 1. Fig. 1. The scaffolds before HeLa cell seedings in DMEM (1x)+ GlutaMAX with FBS and Pen Strep media. a) Freshly seeded HeLa cells, b) 4 days HeLa cells seedings in DMEM (1x)+ GlutaMAX with FBS and Pen Strep media.

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