Tailoring iPSC germ layer differentiation with extracellular microenvironment mechanical and topographical cues

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Induced pluripotent stem cells (iPSCs) have the potential to differentiate into any cell type, making them a promising tool for tissue engineering and regenerative medicine. The efficiency and specificity of iPSC differentiation can be improved by incorporating extracellular microenvironment mechanical and topographical cues. The extracellular matrix (ECM) surrounding cells provides physical and biochemical signals that can influence cell behaviour. During embryonic development, the formation of different germ layers is tightly regulated by complex signalling pathways and spatial cues, which are influenced by the position and orientation of neighbouring cells and tissues. Similarly, in-vitro iPSC differentiation can be enhanced by controlling the spatial organization of iPSCs within the extracellular microenvironment [1,2].

The emergence of 3D bioprinting techniques, opened the door to new opportunities for recreating in-vitro the natural 3D microenvironment surrounding cells [3].

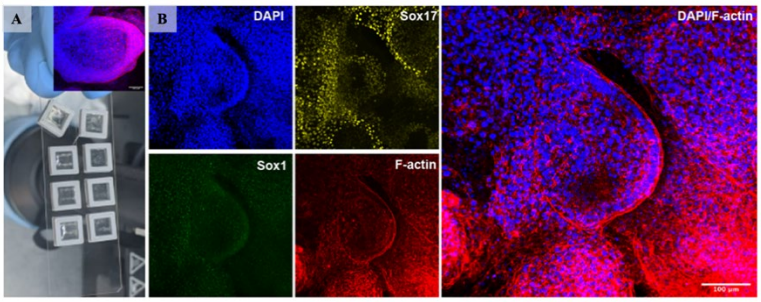

Figure 1: Optical image of printed constructs (A) and immunofluorescenc stained samples for germ layer specific markers (Sox17 for endoderm specification and Sox1 for ectoderm formation) and F-actin (B).

In this study, human iPSCs were deposited in a controlled spatial and temporal manner within a jammed microgel suspension composed of gels with different microgel sizes and porosity, previously developed in our group [4–6]. The iPSC printing in the microgel suspension demonstrated the successful deposition of high-density iPSC aggregates, which are known to be very sensitive cells that can be affected by external physical or biochemical conditions, potentially leading to a loss of viability, pluripotency and differentiation potential (**Figure 1, A**). Surprisingly, the physical properties of the microgel material were able to tune germ layer specification without the need for any biochemical factors (**Figure 1, B).**

References:

[1] S. Lenzini, D. Devine and J. W. Shin, *Front. Bioeng. Biotechnol.*, 2019, **7**, 1–9; [2] L. E. Dickinson, S. Kusuma and S. Gerecht, 2011, 36–49; [3] S. Romanazzo, S. Nemec and I. Roohani, 2019, **1**, 1–18; [4] S. Romanazzo, T. G. Molley, S. Nemec, K. Lin, R. Sheikh, J. J. Gooding, B. Wan, Q. Li, K. A. Kilian and I. Roohani, *Adv. Funct. Mater.*,2021; [5] T. G. Molley, T. tyng Hung and K. A. Kilian, *Adv. Healthc. Mater.*, 2022, **2201122**, 1–1; [6] G. K. Jalandhra, T. G. olley, T. tyng Hung, I. Roohani and K. A. Kilian, *Act a Biomater.*, 2022, **156**, 75–87.