Macromolecular Crowding Meets Cell-Sheet Tissue Engineering: New Concepts in Regenerative Medicine

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Statement of Purpose: Advancements in cell biology and polymer chemistry enabled the development of scaffold-free substitutes, a technology termed Tissue Engineering by Self-Assembly (1). Despite the efficacious in vitro and in vivo results to-date, very few products have been commercialised, primarily due to the prolonged culture time required to develop an implantable device. For example, the production of a corneal stromal layer requires approximately 84 days (2), whilst a blood vessel over 18 weeks (3). It was recently shown that macromolecular crowding (MMC) enhances the deposition of extracellular matrix (4). Herein, the influence of crowding molecules on matrix deposition and the potential of this technology in Tissue Engineering by Self-Assembly was investigated using human fibroblasts.

Materials and Methods: Human primary cells [e.g. lung (WI-38) and skin (WS-1) fibroblasts] were cultured under MMC (e.g. 100µg/ml dextran sulphate (DxS); 100µg/ml polysodium-4-styrene sulfonate (PSS); 37.5mg/ml Ficoll™ 70 and 25mg/ml Ficoll™ 400; 75µg/ml carrageenan (CR) and 100 µl/ml sepharose-CL) and various FBS and HS concentrations (0.0 to 10%). Deposition of extracellular matrix proteins was analysed by SDS-PAGE and immunocytochemistry (ICC). The influence of crowders on cell viability was evaluated using flow cytometry. To lift-up the cell-matrix-rich sheet, NIPAM thermal-responsive polymers were developed. An extensive proteomic study was carried out using mass spectrometry and validated by ICC to comprehend the expression and deposition of proteins.

Results & Discussion:

Figure 1: Densitometric analysis of SDS-PAGE demonstrated that MMC significantly increase type I collagen deposition ($p<0.0001$) at all tested serum concentrations. The fibroblasts deposited maximum collagen I at day 2 with 0.5% serum.

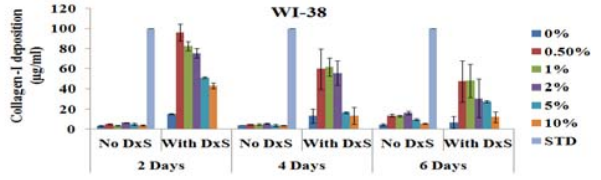


Figure 2: Densitometric analysis of SDS-PAGE demonstrated that in the presence of HS, as opposed to FBS, significantly higher ($p<0.001$) collagen I was deposited by both skin and lung fibroblasts.

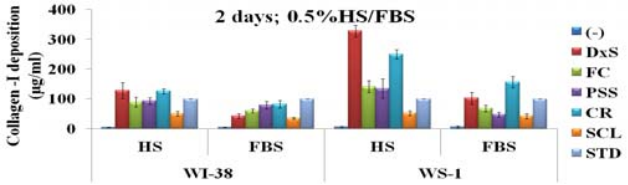


Figure 3: ICC analysis confirmed the enhanced deposition of collagen I and its co-localisation with fibronectin in the presence of crowders.

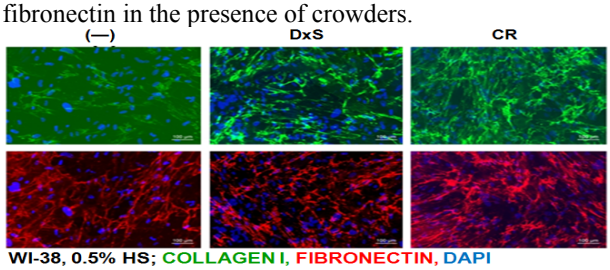


Figure 4: Culture plate coated with 65% N-Isopropylacrylamide: 35% N-tert butyl acrylamide facilitated lifting-up a rich in ECM cell-sheet.

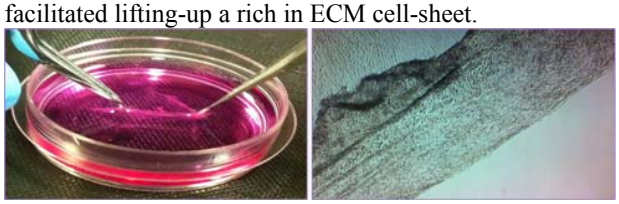
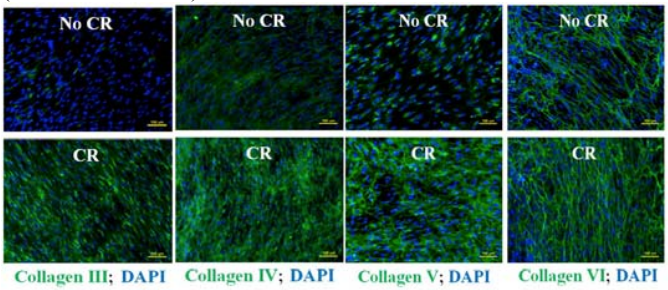


Figure 5: Complementary ICC for mass spectrometry validation confirmed the enhanced deposition under crowding of collagens (III, IV, V and VI) and other ECM molecules (e.g. laminin, fibronectin, hyaluronic acid, decorin, and lysyl oxidase), without changing expression of elastin, tubulin, α-actin, epithelial keratin and IL-10 (results not shown).



Conclusions: All in all, these data indicate that MMC facilitates the production of rich in ECM cell-sheets without any modification of basic cellular properties.

References:

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