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Bioprinting of Smooth Muscle Constructs for Urethral Tissue Engineering

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INTRODUCTION: The aim of urethral tissue-engineering is to develop biomaterial- and cell-based therapies for the repair of urethral defects. In recent years, bioprinting has emerged as a promising approach to overcome the poor cell infiltration associated with conventional scaffold-based methods. In addition, bioprinting would enable the fabrication of hierarchical cell-laden structures that mimic the architecture of native urethral tissue. One of the key challenges remains finding biomaterials with optimal printability, which could support proper assembly and maturation of cells.

METHODS: Gelatin methacrylate (GelMA, CellInk) and pure collagen (Lifeink 200, Advanced Biomatrix) were assessed in regards to their ability to support bioprinting of human urothelial smooth muscle cells (Provitro). Ring-shaped constructs (6 mm in diameter, 1 mm in height) were printed using an extrusion-based bioprinter (BioX, CellInk). Following bioprinting, live/dead and metabolic assays were used to evaluate cell viability and proliferation. Cell distribution and morphology were determined by hematoxylin and eosin staining. Upon a 10-day induction period, the maturation of the encapsulated cells was assessed by immunofluorescence staining and semi-quantitative RT-PCR.

RESULTS: Both GelMA and collagen constructs showed a high percentage of cell survival (>80%). Cells adopted a spindle-like morphology and displayed a sustained growth rate over a 5-day period. Morphological analysis revealed that cell density increased over time, mainly in the periphery of the constructs. After induction to differentiation, cells showed enhanced expression of smooth muscle actin, which was accompanied by an increased transcriptional activity of ACTA2 (α -smooth muscle actin), CALD1 (caldesmon) and CNN1 (calponin).

DISCUSSION & CONCLUSIONS: The high cell biocompatibility of GelMA and collagen-based bioinks can be explained by the presence of RGD motifs that support cellular attachment, migration and growth. Bioprinting with collagen appears more straightforward than GelMA, as it does not require an additional photo-crosslinking step. However, the optimal mechanical properties of the constructs still need to be determined considering its key role in controlling the contractile cell phenotype. The results of this work, although preliminary, suggest that both GelMA and pure collagen constitute suitable bioink platforms for the fabrication of cell-laden constructs for urethral tissue engineering.

Keywords: Biofabrication, Differentiation