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MYOGENIC AND EPITHELIOGENIC DIFFERENTIATION OF ADIPOSE AND BUCCAL MUCOSAL STEM CELLS FOR ARTIFICIAL URETHRA CONSTRUCTION

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The urethra is crucial in the urinary system, facilitating urine flow from the bladder to the external environment. Repairing urethral damage is essential for restoring normal urinary functions and preventing long-term complications[1]. Current treatments typically involve surgical procedures that often require multiple interventions. Our goal is to enhance these treatments using 3D printing technology to create an artificial urethral tissue. This tissue aims to provide an ideal environment for cell expansion, migration, and differentiation into a functional tissue.

Our artificial tissueś foundation is a 3D scaffold composed of Gelatin Methacrylate (GelMA) and Silk Fibroin (SF). This scaffold forms a hydrogel with a firm structure when sonicated to denature the silk fibroin and exposed to UV to polymerize GelMA[2]. For the cellular aspect, itś necessary to mimic the urethraś natural structure, which consists of an epithelial wall surrounded by a muscular wall. Our previous work showed that rabbit adipose stem cells (RASC) are suitable for myogenic differentiation. And our recent findings indicate that RASC can effectively differentiate into myogenic-like cells in the GelMA-SF 3D environment as well. This was confirmed by measuring expressions of myogenic differentiation markers *Acta2* and *Cald1*, which showed increased expression levels in differentiated RASC within the GelMA-SF hydrogel. Additionally, alpha-SMA was immunocytochemically stained in RASC, showing higher protein expression in differentiated cells compared to undifferentiated ones. These results suggest successful myogenic-like cell differentiation of RASC cells hosted in a GelMA-SF hydrogel[3].

For epithelial differentiation, we selected rabbit buccal mucosa stem cells (RBMC). Their differentiation, conducted in 2D conditions, showed reduced *Ck14* gene expression and proliferation rates after 5 and 10 days, which is an indication of epithelial differentiation[4]. A cell morphology analysis of images, acquired by immunocytochemical staining of CK14, indicated that differentiated RBMC cells grew significantly larger which is consistent with epithelial differentiation progression. Additionally from the immunocytochemistry images cell density was evaluated, the results from these combined with the results of an MTT test showed a reduced proliferative rate of the differentiated cells. These findings suggest a successful transformation of RBMC cells into non-proliferative epithelial cells.

In summary, our research demonstrates that RASC in 3D GelMA-SF hydrogels can successfully differentiate into myogenic-like cells, and RBMC cells are capable of epithelial differentiation. Future studies will focus on combining these differentiated cell lines to replicate urethral tissues.

[1] Stein DM, et al. A geographic analysis of male urethral stricture aetiology and location. 2012 Dec 18
[2] Wenqian Xiao, et al. Cell-laden interpenetrating network hydrogels formed from methacrylated gelatin and silk fibroin via a combination of sonication and photocrosslinking approaches. 2019 Jun 1
[3] Helms F, et al. Complete Myogenic Differentiation of Adipogenic Stem Cells Requires Both Biochemical and Mechanical Stimulation. 2019 Feb 27
[4] Alam H, et al. Novel function of keratins 5 and 14 in proliferation and differentiation of stratified epithelial cells. 2011 Nov 1