In vitro Response of Human Buccal Epithelial Cells to a Bladder Patch for Vesicovaginal Fistula Repair

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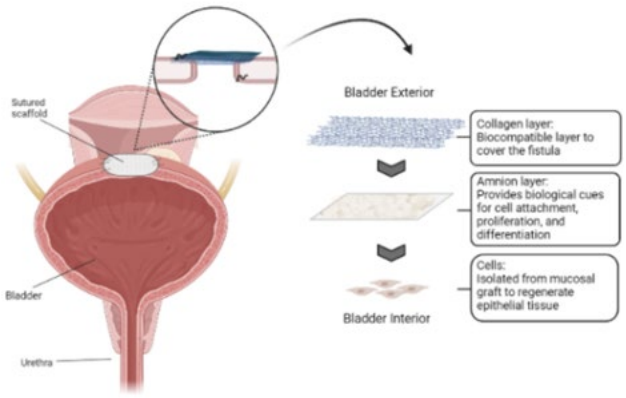
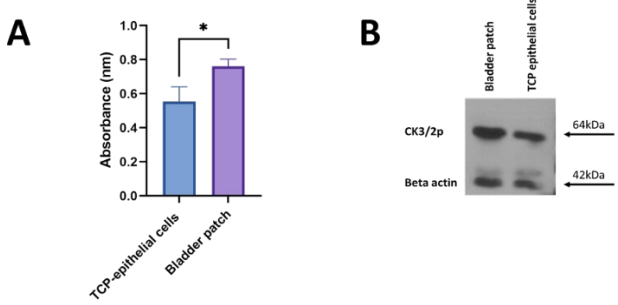
Introduction: Replacing bladder tissue with a functional equivalent remains one of the most challenging problems for conditions such as iatrogenic injuries, trauma, or fistula in reconstructive urology [1]. Nearly 3.5 million women worldwide currently live with an obstetric-related vesicovaginal (VVF) fistula [2]. Approximately 100,000 new cases develop each year, primarily affecting women in low-resource countries. Amnion tissue has been found to be not only regenerative, but also non-allogenic and highly sustainable in the bladder and in the vagina. Utilization of naturally derived biomaterials such as amnion and collagen can provide biochemical cues for cell attachment, growth, and proliferation. Additionally, oral epithelial cells represent a viable source of cells for clinical applications as they can be collected from small biopsies and expanded for tissue regeneration. Development of a durable cell-seeded hybrid scaffold that would not require the extensive dissection or suturing required during standard surgical repair of a VVF fistula could be a potential treatment option for patients with a VVF (Fig.1).



Materials and methods: Human oral epithelial cells were harvested from buccal grafts under an IRB-approved protocol, and their phenotype was assessed by immunocytochemistry using a fluorophore-conjugated antibody against CK3/2p. Collagen sheets were fabricated using an electrochemical compaction method with two planar electrodes[3] and crosslinked using genipin (2%, %90 ethanol). A gluing solution containing sodium hyaluronate, dextran and bovine serum albumin was used to attach human amniotic membrane isolated from the placenta to the collagen sheets. Adhesion between the amnion and collagen sheet layers was observed using scanning electron microscopy (SEM). Oral epithelial cells were seeded on the amnion side and cultured for three days. Cell proliferation on seeded collagen sheets was assessed using MTT assay. Western blot for CK3/2p was conducted to determine the phenotype of seeded cells at 72 hours. Phenotype was compared against tissue culture plate (TCP).

Results: Human oral epithelial cells stained positive for CK3/2p. SEM showed a connection between layers within composite bladder patch. Epithelial cells displayed increased cell proliferation when seeded on bladder patch compared to epithelial cells that are seeded on the culture plate ($p<0.05$) (Fig.2A). Protein expression highlighted the presence of CK3/2p at 72 hours following seeding on a bladder patch (Fig.2B).



Conclusions: Multiphasic bladder patch supports sufficient epithelial cell attachment and survival suitable for implantation. Protein expression results suggest that a multiphasic bladder patch sustains the epithelial cell phenotype and increases the proliferation rate. The proposed bioengineered bladder patch is novel, can be utilized in conjunction with epithelial cells and has tremendous potential for regenerative medicine-based repair of bladder tissue. We are in the process of testing this patch for biocompatibility and mechanical properties in animal studies for long-term safety *in vivo*. Another focus of future studies will be comparing cell phenotype between with and without the amnion layer to determine bioinductive effects of the amnion.

References:

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