Localized Treatment of Interstitial Cystitis Using a Novel Cell Surface Engineering Approach

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Introduction

Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS)

- Five times more prevalent in women, affecting around 3.3 to 7.9 million women in US1
- Symptoms: bladder pain, frequency, urgency, and incontinence2
- Only short-term symptom relief treatment, such as heparin bladder instillation, available due to unclear etiology3
- Glycosaminoglycan (GAG) layer or glycolalcyx disruption, neurogenic inflammation, autoimmune disorders are among the most prominent causes of IC/BPS1,2
- GAG layer disruption and diffusion of harmful substances leads to nerve ending stimulation and neurogenic inflammation, followed by immune cell recruitment and more damage to GAG layer1,2,3

Methods

In vitro Injury Model

(a) Normal GAG Layer

(b) Cell Surface Engineering with LPGSQ

In vitro Injury Model

- In vitro injury model established using TNF-α or proline-sulfate (PS) on HTB4 human bladder tissue and this model produces an inflammation-based injury, whereas PS only sheds the GAG layer from the surface
- Injury model confirmation done via flow cytometry to quantify the change in GAG layer content.
- Transepithelial protein passage assay used to assess the effect of insult on transepithelial permeability

Cell Surface Engineering with LPGSQ

- TransGase mediated cell surface engineering of HTB4 cells using LPGSQ (Mw of LPG backbone = 15 kDa) to recover GAG layer functionality and reduce inflammation
- Structural recovery of GAG layer in engineered cells evaluated by GAG content measurement via flow cytometry, and GAG functional recovery characterized by wound healing test and measurement of pro-inflammatory marker, IL-6, via ELISA

Figure 1. Structure of normal and dysfunctional GAG layer. GAG layer consists of glycoproteins and proteoglycans. Sugars in the GAG layer include sulfated sugars, such as chondroitin sulfate and heparan sulfate, and non-sulfated sugars, such as hyaluronic acid. Disruption of GAG layer can lead to diffusion of harmful substances to underlying tissue and cause symptoms associated with IC/BPS.1,3

Figure 2. Schematic representation of (a) in vitro injury model for GAG layer disruption, (b) TransGase mediated CSE approach using sulfated linear polyglycerol with a peptide linker (LPGSQ) to rescue the injured GAG layer and the architecture of LPGSQ.

Figure 3. Effect of TNF-α or PS treatment on transepithelial protein passage permeability. Transepithelial permeability was assessed by measuring the passage of labeled bovine serum albumin (BSA) as a model protein, through HTB4 monolayer in a well plate with transwell inserts. Measurement was conducted following TNF-α or PS treatment for (a) 24 hours.

Figure 4. Effect of CSE with LPGSQ on GAG layer injury inhibition. To quantify GAG layer injury, sugars on GAG layer were stained with wheat germ agglutinin (WGA) and quantified using flow cytometry.

Figure 5. Effect of CSE with LPGSQ on inflammation. The effect of LPGSQ cell surface engineering on IL-6 concentration, as a pro-inflammatory cytokine, was studied using ELISA in two different insult models. The models include (a) only TNF-α treatment and (b) combination of TNF-α and PS treatment.

Figure 6. Effect of LPGSQ modification on HTB4 cells wound healing. Wound healing was studied by scratch test following LPGSQ modification and imaging was conducted via brightfield microscopy.

Figure 7. Architecture of immunomodulatory polymer conjugate with Sialic acid sugar conjugate (purple).

Conclusions and Future Directions

- Cell surface engineering of HTB4 cells using sulfated linear polyglycerol leads to reduced inflammation upon injury, but no change in GAG layer shedding and wound healing was observed.
- Transepithelial permeability decreased slightly after 24 hours of insult, whereas transepithelial permeability is known to decrease considerably upon GAG shedding. Thus, an improved permeability model should be established for future work.
- Other biomarkers, including pro- and anti-inflammatory cytokines and tight junction proteins should be studied to further validate the effect of LPGSQ treatment.
- Interaction of engineered epithelial cells and immune cells should be studied in a suitable co-culture model.
- To better model the glycoproteins in GAG layer, larger polymer conjugates (up to 1 MDA) with more suitable sugar conjugates should be synthesized and tested.

References