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 US[1]
- Symptoms: bladder pain, frequency, urgency, and incontinence[2]
- Only **short-term** symptom relief treatment, such as heparin bladder instillation, available due to unclear etiology[2]
- **Glycosaminoglycan (GAG) layer or glycocalyx disruption**, neurogenic inflammation, autoimmune disorders are among the most prominent causes of IC/BPS[2,3]
- **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 layer[2,3]

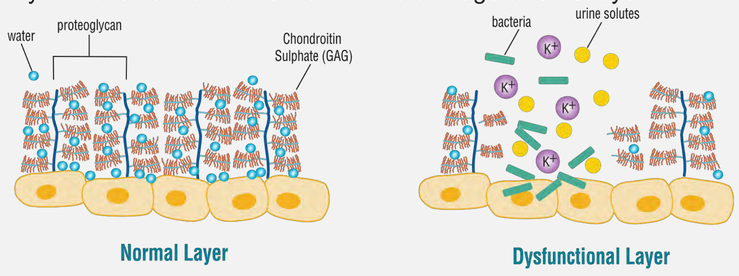

Figure 1. Structure of normal and dysfunctional GAG layer. GAG layer consists of glycoproteins and proteoglycans. Sugars in the GAG layer include sulfated sugars, such as chondroitin sulfate and heparan sulfate , and non-sulfated sugars, such as hyaluronic acid. Disruption of GAG layer can lead to diffusion of harmful substances to underlying tissue and cause symptoms associated with IC/BPS. [4,5]

Objectives

1. To establish a reliable *in vitro* cell injury model to study GAG layer disruption in IC/BPS.

2. To study whether enzymatic cell surface engineering (CSE) of epithelial cells with immunomodulatory polymer conjugates could recover GAG layer functionality and reduce inflammation.

Methods

(a) **In vitro Injury Model**

(b) **Cell Surface Engineering with LPGSQ**

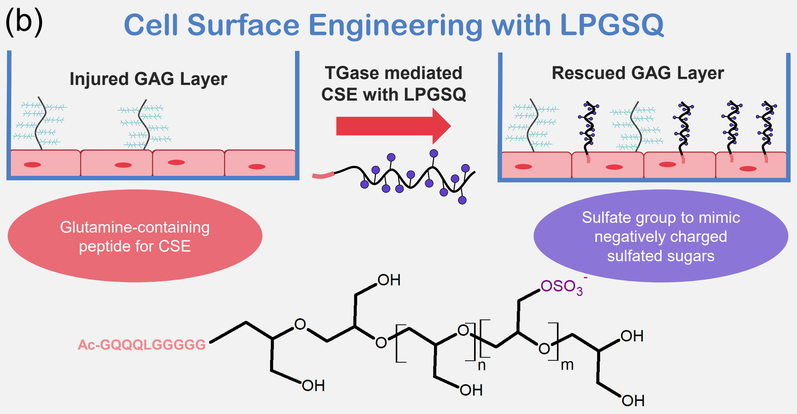

Figure 2. Schematic representation of (a) *in vitro* injury model for GAG layer disruption, (b) TGase mediated CSE approach using sulfated linear polyglycerol with a peptide linker (LPGSQ) to rescue the injured GAG layer and the architecture of LPGSQ.

In vitro Injury Model

- *In vitro* injury model established using TNF-α or protamine sulfate (PS) on HTB4 human bladder cells. TNF-α induces 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

- TGase mediated cell surface engineering of HTB4 cells using LPGSQ (M_w 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

Results

Transepithelial permeability decreases after 24 hours due to insult-mediated GAG layer shedding

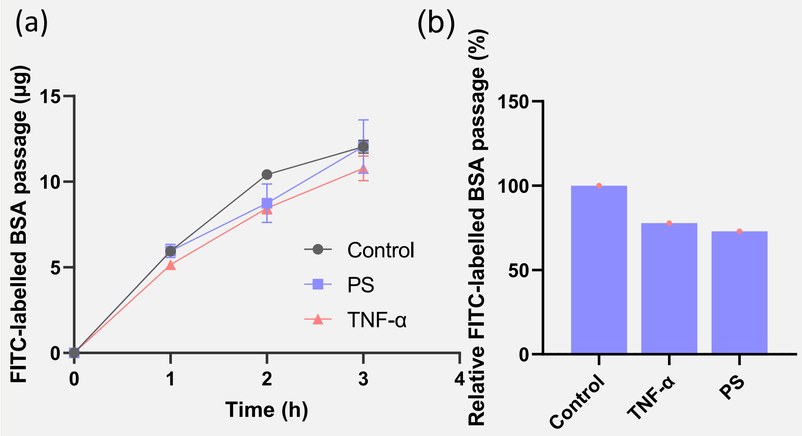

Figure 3. Effect of TNF-α or PS treatment on transepithelial protein passage permeability. Transepithelial permeability was assessed by measuring the passage of labelled bovine serum albumin (BSA), as a model protein, through HTB4 monolayer in a well plate with transwell inserts. Measurement was conducted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

Insult mediated GAG layer shedding was not inhibited by LPGSQ modification

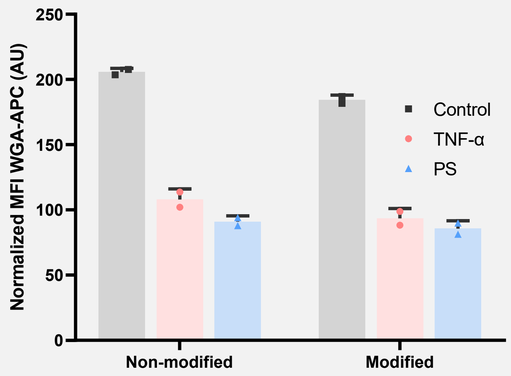

Figure 4. Effect of CSE with LPGSQ on GAG layer injury inhibition. To quantify GAG layer injury, sugars on GAG layer were stained with wheat germ agglutinin (WGA) and quantified using flow cytometry.

Inflammation decreases due to LPGSQ modification

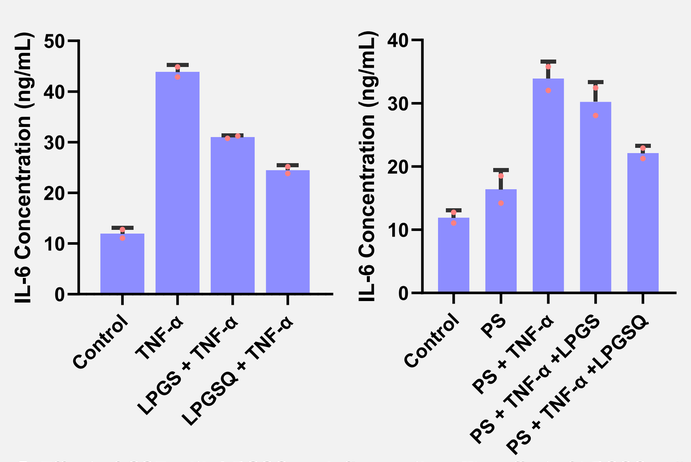

Figure 5. Effect of CSE with LPGSQ on inflammation. The effect of LPGSQ cell surface engineering on IL-6 concentration, as a pro-inflammatory cytokine, was studied using ELISA in two different insult models. The models include (a) only TNF-α treatment and (b) combination of TNF-α and PS treatment.

Wound healing behaviour of HTB4 cells did not change following LPGSQ modification

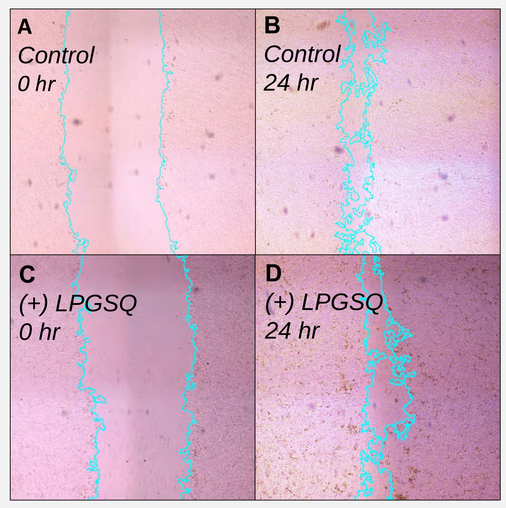

Figure 6. Effect of LPGSQ modification on HTB4 cells wound healing. Wound healing was studied by scratch test following LPGS-Q modification and imaging was conducted via brightfield microscopy.

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.
- Immunomodulatory polymer conjugates could play a key role in developing a curative long-term treatment for IC/BPS, which can have a significant impact on the quality of life of IC/BPS patients and eliminate the need for invasive instillation treatments.

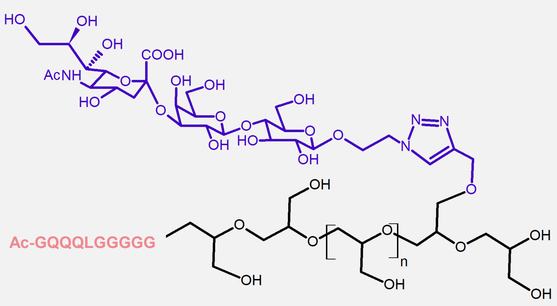

Figure 7. Architecture of immunomodulatory polymer conjugate with Sia3Lac sugar conjugate (purple).

References

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Acknowledgements

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