ORIGINAL ARTICLE

# SuperGAG biopolymers for treatment of excessive bladder permeability

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## Abstract

Few therapeutic options exist for treatment of IC/BPS. A novel high MW GAG biopolymer ("SuperGAG") was synthesized by controlled oligomerization of CS, purified by TFF and characterized by SEC-MALLS and 1H-NMR spectroscopy. The modified GAG biopolymer was tested in an OVX female rat model in which bladder permeability was induced by a 10-minute intravesicular treatment with dilute (1 mg/ml) protamine sulfate and measured by classical Ussing Chamber TEER measurements following treatment with SuperGAG, chondroitin sulfate, or saline. The effect on abrogating the abdominal pain response was assessed using von Frey filaments. The SuperGAG biopolymer was then investigated in a second, genetically modified mouse model (URO-MCP1) that increasingly is accepted as a model for IC/BPS. Permeability was induced with a brief exposure to a sub-noxious dose of LPS and was quantified using contrast-enhanced MRI (CE-MRI). The SuperGAG biopolymer restored impermeability to normal levels in the OVX rat model as measured by TEER in the Ussing chamber and reduced the abdominal pain response arising from induced permeability. Evaluation in the URO-MCP1 mouse model also showed restoration of bladder impermeability and showed the utility of CE-MRI imaging for evaluating the efficacy of agents to restore bladder impermeability. We conclude novel high MW SuperGAG biopolymers are effective in restoring urothelial impermeability and reducing pain produced by loss of the GAG layer on the urothelium. SuperGAG biopolymers could offer a novel and effective new therapy for IC/BPS, particularly if combined with MRI to assess the efficacy of the therapy.

**KEYWORDS**
electrophysiology, glycosaminoglycans, interstitial cystitis/bladder pain syndrome, intravesical administration, magnetic resonance imaging, neurogenic inflammation, permeability, urinary bladder, urothelium

# 1 | INTRODUCTION

Interstitial cystitis (IC), or bladder pain syndrome (BPS), is a variably expressed and possibly heterogeneous chronic disease characterized by frequent urination, increased urgency, and pain associated with bladder filling.[1] Diagnosis is based on clinical criteria,[2-6] and with current definitions as many as 4 to 12 million people in the United States, mostly women, could be affected.[7,8] IC/BPS shows a high co-morbidity with other pelvic pain disorders; some 30% to 50% of irritable bowel syndrome (IBS) patients also exhibit symptoms of IC/BPS, while up to 40% of patients with IC/BPS also exhibit symptoms of IBS.[9,10] These high comorbidities indicate crosstalk among the lower abdominal organs and that treatment of IC/BPS could favorably affect the comorbidities as well. The costs to the health care system and economy run into the hundreds of millions to billions of dollars, and the impact on patient lives can be devastating.[11,12]

Therapeutic options for treating IC/BPS are generally limited to treating pain or attempting to restore impermeability with GAG-replenishment therapy.[2,13] Epithelial permeability in IC/BPS is a major etiologic factor.[14,15] Histologic evidence in both humans and cats shows loss of the luminal (umbrella cells) layer of cells that contain the tight junctions and the GAG layer that are the main defenses against urine solute penetration of the bladder, leaving the bladder surface permeable to urinary solutes.[16-24] Loss of the GAG layer from enzymatic digestion alone in animal models also induces bladder permeability [25] indicating that an intact GAG layer comprises the major barrier to penetration by urinary solutes. Treating pain is palliative and does not restore the impermeability of the urothelium.

In GAG-replenishment therapy, solutions of linear GAG biopolymers (e.g., hyaluronan, CS, pentosan polysulfate or heparin) are infused directly into the bladder to replenish the defective GAG layer and restore impermeability. Partial efficacy has been demonstrated in many clinical trials,[26] and in animal models of bladder permeability intravesical GAG treatment restores normal impermeability and inhibits recruitment of inflammatory cells.[27] The therapeutic most recently approved by the FDA for IC/BPS was Elmiron® in 1996. An oral drug with only 6% bioavailability, Elmiron® is thought to augment the GAG layer when excreted, but clinical response is limited.[28] The clinical effectiveness of linear GAGs are temporary and response rates rarely exceed 50% to 60%.[29]

The mechanism driving the loss of impermeability, and whether its loss is primary or secondary, are both unclear. Penetration of potassium ions induces pain by activating sensory nerves innervating the bladder.[30] Evidence suggests that permeability can be induced both endogenously through neural connections,[31-35] possibly modulated by inflammatory cells, and from cationic substances in the urine[36,37] exacerbated by loss of cation scavengers.[38] A vicious cycle in which loss of impermeability becomes permanent with sensory nerve upregulation leads to a chronic pain syndrome. Interruption of this vicious cycle may be the key to restoration of normal bladder impermeability and a key factor for therapeutic effectiveness.

The GAGs of the normal urothelium are actually present as PGs[16] that form a thick layer of bound water with a very high anionic charge density.[39] The single chain GAGs used therapeutically are not able to mimic the three-dimensional sulfated GAG environment provided by PGs on the surface of the native urothelium. Our hypothesis is that the efficacy of GAG replenishment is limited because the currently available generic linear biopolymers that are used do not adequately mimic the native layer of protective PGs. In this communication, we test the hypothesis that a novel class of branched, high MW, sulfated GAGs (known as "SuperGAGs") with a reticulated three-dimensional structure that more closely mimics natural PGs will more effectively restore impermeability and reduce pain than the currently used clinically linear sulfated GAGs (CS), or hyaluronan in validated animal models.

# 2 | MATERIALS AND METHODS

## 2.1 | Materials

CS was obtained from Bioiberica (Barcelona, Spain, EP Injectable grade). DVS was purchased from ACROS Organics (99% purity). The equivalent weight of the disodium chondroitin 4-sulfate structural repeat unit is 503.35 g/equiv. ($C_{14}H_{19}O_{14}SNa_2$), and the hydroxyl equivalent weight is 503.35/3 = 167.78 g/OH equiv. Except as noted, all chemicals were obtained from Sigma-Aldrich (St. Louis, MO) and were prepared fresh prior to use. Protamine sulfate was dissolved in saline at 1 mg/ml. The composition of Krebs solution was 120 mM NaCl, 6 mM KCl, 1.2 mM $MgCl_2$, 1.2 mM $H_2PO_4$, 2.5 mM $CaCl_2$, 14.4 mM $NaHCO_3$, and 11.5 mM glucose.

OVX female rats were purchased from Charles River Laboratories (Wilmington, MA). A breeding pair of URO-MCP-1 transgenic mice were obtained from Dr. Y. Luo of the University of Iowa and offspring were raised at the OMRF animal facility. For ease in catheterization only female mice were used. All animal protocols were approved by the IACUCs at the OUHSC and OMRF animal facilities. The animals had free access to food and water and were acclimated to facility housing for a minimum of one week before experimentation. Animals were euthanized by cervical dislocation following induction of deep anesthesia with Isoflurane-oxygen.

## 2.2 | General procedure for synthesis, purification, and characterization of SuperGAG biopolymers

Synthesis of the SuperGAG was performed using a staged addition method as described previously.[40,41] For example, for the synthesis of SuperGAG CS (0.720 g, 4.0 meq hydroxyl groups) and sodium chloride (189 mg) were dissolved in 19.44 ml deionized water in a 50 ml reaction vessel. A clear colorless solution was obtained. DVS (0.473 g, 402 µl, 4.0 mmol) was added volumetrically with a microliter pipette. After gentle mixing, the solution was clear and

colorless. Reaction was initiated by the addition of 2.16 ml of 1.0 N NaOH. With the addition of NaOH, the solution immediately became pale yellow in color and remained clear. The reaction was gently mixed on a rotisserie. After 15 minutes, additional solid sodium CS was added (1.680 g, 9.35 meq hydroxyl groups), and the reaction mixture was agitated on a rotisserie. The reaction solution became slightly viscous but remained clear and fluid. Ninety minutes after initiation by NaOH, the reaction was quenched by adding 2.16 ml of 1.0 N HCl. The clear fluid reaction mixture was diluted with PBS to a total volume of 160 ml. The diluted reaction mixture was easily filtered through a 0.45 um PVDF syringe filter.

Purification of the SuperGAG biopolymer was achieved using tangential flow filtration (TFF). A Spectrum Lab KR2i TFF system was used with a 250 ml feed reservoir and a 20-cm hollow fiber filter module containing modified polyethersulfone filter fibers (1 mm diameter, 100 kDa MWCO, 75 cm$^2$ total surface area). An 80 ml portion of the diluted reaction mixture described above was loaded into the feed reservoir. The tangential flow filtration was run in dialysis mode with continuous replenishment with PBS until five volumes (400 ml) of permeate was collected. The TFF was then continued in desalting mode by replenishing with DI water instead of PBS and continuing filtration until an additional five volumes of permeate (400 ml) was obtained. Replenishment was then halted briefly to concentrate the product. The purified retentate was then collected and dried by lyophilization for at least 72 hours, yielding 0.48 g of purified high MW product (38% yield relative to starting CS weight) as a white fluffy solid. Although CS is stable for years at room temperature, the product was stored at −80°C.

The molecular weight of SuperGAG composition and CS was measured by SEC-MALLS performed at an independent, ISO 17025 accredited analytical laboratory. Experimental conditions for SEC-MALLS are available from the authors. Identical duplicate chromatograms were obtained for each material. The reported MW values are the average of the duplicate runs. 1H-NMR spectra were obtained on SuperGAG solutions in D$_2$O solution (15 mg/ml) using a JEOL 400 MHz NMR with DELTA 5.3.0 processing software.

## 2.3 | Animal model methods

Two animal models were used. A rat model described in detail previously[25,42-45] was used to confirm that the SuperGAG restores impermeability using the TEER "gold standard." This same model was used to confirm that restoring bladder impermeability abrogates the abdominal pain result. The restoration of impermeability also was confirmed in a transgenic mouse model that is receiving increasing acceptance as a model for IC/BPS.[46] using MRI as developed in our labs. The mouse bladder is too small for reliable TEER measurements.

Beginning at 9 a.m. (Day 0) OVX female Sprague-Dawley rats were anesthetized with isoflurane-oxygen, catheterized with a 24 ga. intravenous catheter (Terumo Medical) and 400µL of 1 mg/ml protamine sulfate was instilled into the bladder. As discussed in our previous publications using this model, this dose of protamine sulfate is 1/10th that usually used and produces minimal visible urothelial damage.[18-20] The protamine sulfate was removed after 10 minutes and the animals were returned to their cages. Beginning at 9 a.m. the following day (Day 1), the animals were again anesthetized with isoflurane-oxygen and were instilled with either 400µl of 20 mg/ml SuperGAG in saline or saline alone as a control. For comparison because CS is used clinically, a set of animals were instilled with 400µl of 20 mg/ml CS. Because the interaction with the bladder wall is likely electrostatic through the negative charges on the GAG chains, equal weights of SuperGAG and CS were compared. Beginning at 12 p.m. the rats were euthanized, and the bladder was isolated. The bladder was opened and mounted whole, urothelium side up, using a small chamber clamped over the urothelium. The electrophysiologic variables of potential difference (PD) and short circuit current (Isc) were measured to assess TEER in both bladder and colon as described previously.[42] Sham-treated animals were instilled with saline instead of protamine sulfate or SuperGAG/CS to account for any artifacts of catheter damage.

Bladder sensitivity was assessed using von Frey filaments applied to the suprapubic region in the rat model. Female OVX rats were infused intravesically with protamine sulfate (1 mg/ml), and 24 hours later treated with i) vehicle, ii) CS (20 mg/ml) or iii) SuperGAG (20 mg/ml). Controls consisted of animals receiving a sham instillation instead of protamine sulfate or any treatment. Bladder sensitivity was assessed 3 hours later. Each von Frey filament was applied for 1–2 seconds for 10 applications. The filaments were tested from lowest to highest force. Sharp retraction of the abdomen, immediate licking or grooming or jumping was considered a positive response.

The URO-MCP-1 transgenic model was described previously.[46] Cystitis with accompanying permeability were induced by intravesical administration of LPS at a sub-noxious dose of 1 µg of LPS in 100 µl of saline (Day 0, 9 a.m.). One control group was administered saline (100 µl) only (saline URO-MCP-1). Another control group consisting of wild-type (WT) mice was also administered saline only (saline-WT). Bladders were flushed 3 times with saline to remove any excess LPS 1 hour post-LPS injection. The next day (Day 1, 9 am) animals were treated with CS or SuperGAG (20 mg/ml in saline; 100 µl) or saline and the initial permeability was assessed 3 hours later (Day 1, 12:00 p.m.).

For MRI assessment of permeability, mice were anesthetized and treated as described above, but instead of euthanizing the mice, on Day 1, Day 3, and Day 5 the mice were anesthetized, instilled with the contrast agent Gd-DTPA and placed into the MRI instrument.[45] MRI experiments were conducted on a 7 Tesla 30 cm-bore Bruker Biospec MRI system (Bruker Biospin Corporation, Woodlands, TX, U.S.A.). MRI scans were obtained at 1-, 3-, and 5-days following LPS instillation. For the bladder images, Gd-DTPA (0.034 mM Gd-DTPA diluted to 100 µl in saline), was administered via an intravesical catheter, for visualization of loss of permeability of the bladder urothelium. Bladder CE-MRI signal intensity changes were determined 7 minutes. post-contrast. For all MR images, a T1-weighted RARE (rapid acquisition with relaxation enhancement) MRI pulse sequence was used with the following parameters: repetition time (TR) of

1200 ms, echo time (TE) of 9 ms, a RARE factor of 4, 4 averages, an image slice thickness of 1 mm, a matrix of 256 × 256, a field-of-view (FOV) of 3.5 × 3.5 cm$^2$, and with both motion and fat suppression. Animals were euthanized and the bladders placed in formalin for histopathology.

MRI signal intensities were measured from regions-of-interest (ROIs) within images (4–5 ROIs were taken in high-intensity regions in the bladder periphery along with corresponding regions in saline control animal datasets) displayed on Paravision (v 5.0, Bruker Biospin).[45]

## 2.4 | Statistical analysis

The statistical analyses were performed using GraphPad Prizm (GraphPad Software, LaJolla, CA). Means and standard deviations of data sets were calculated and compared using Student's $t$ test. Pain data were analyzed by two-way ANOVA w/ Bonferroni's multi-comparison post-test. A value of $p < 0.05$ was taken as being statistically significant.
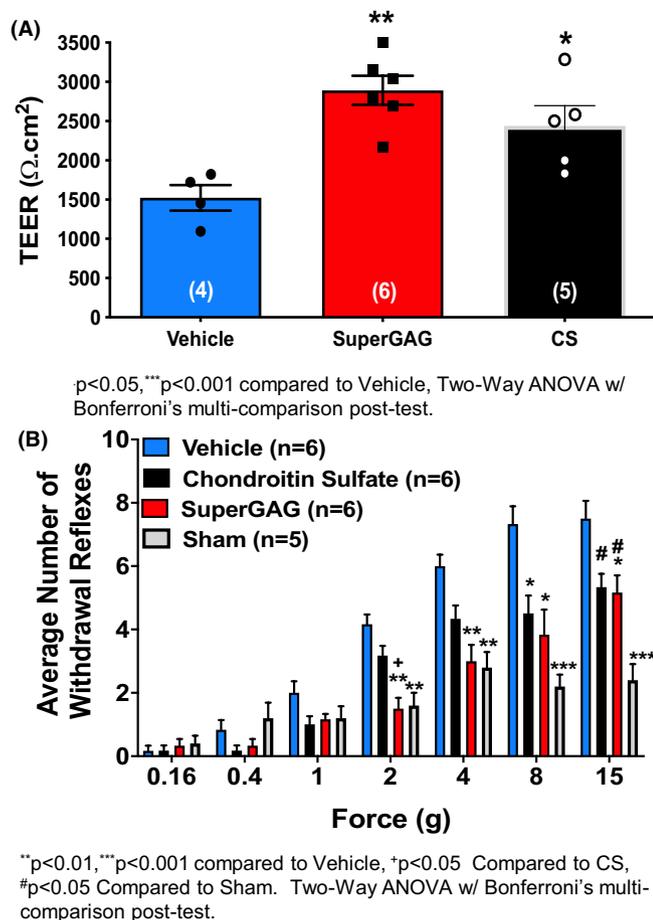
## 3 | RESULTS

The synthesis and properties of SuperGAG compared to the original CS is described in Figure 1. The activation and bond formed linking two CS chains together is illustrated in Figure 1A. Figure 1B illustrates the overall synthesis of a dendritic, proteoglycan-like structure. The resulting SuperGAG biopolymer was highly soluble in water and saline, producing solutions of low to moderate viscosity that was filterable through an 0.2 micron filter. Figure 1C shows the molecular weight distributions of the original CS and the SuperGAG, showing that the size distribution of the product is highly reproducible The molecular weights of the CS and the SuperGAG biopolymer of this study were obtained by SEC-MALLS and are summarized as follows. SuperGAG had a molecular weight of $M_n = 1.94 \times 10^5$, $M_w = 11.2 \times 10^5$ and $M_z = 68.7 \times 10^5$ and a polydispersity index (PDI, $M_w/M_n$) of 5.78. For the CS raw material, the equivalent values were $M_n = 1.17 \times 10^4$, $M_w = 1.37 \times 10^4$, $M_z = 16.7 \times 10^4$ and PDI = 1.17. Although SuperGAG was found to be highly polydisperse, the refractive index and light scattering chromatograms for both biopolymers showed acceptable gaussian peak shapes. The SuperGAG product was comprised of >16 chains based on $M_n$ values. The number of CS monomers incorporated into the SuperGAG can be varied by adjusting synthesis conditions and the concentrations of DVS and CS.

Figure 2A demonstrates that the SuperGAG biopolymer restored the TEER in the rat protamine sulfate model to control values of TEER = 2500 ± 249 Ωcm$^2$.[25,42] There is general agreement that the most significant symptom of IC/BPS is pain. In another experiment, protamine sulfate-treated rats were evaluated for a visceral pain response. Induced bladder permeability produced a strong visceral pain response as measured with von Frey filaments and this pain was significantly abrogated by instillation of SuperGAG (Figure 2B). These data show that acute induction of bladder permeability produced a measurable pain response, and that GAG replacement abrogated this pain response except at the
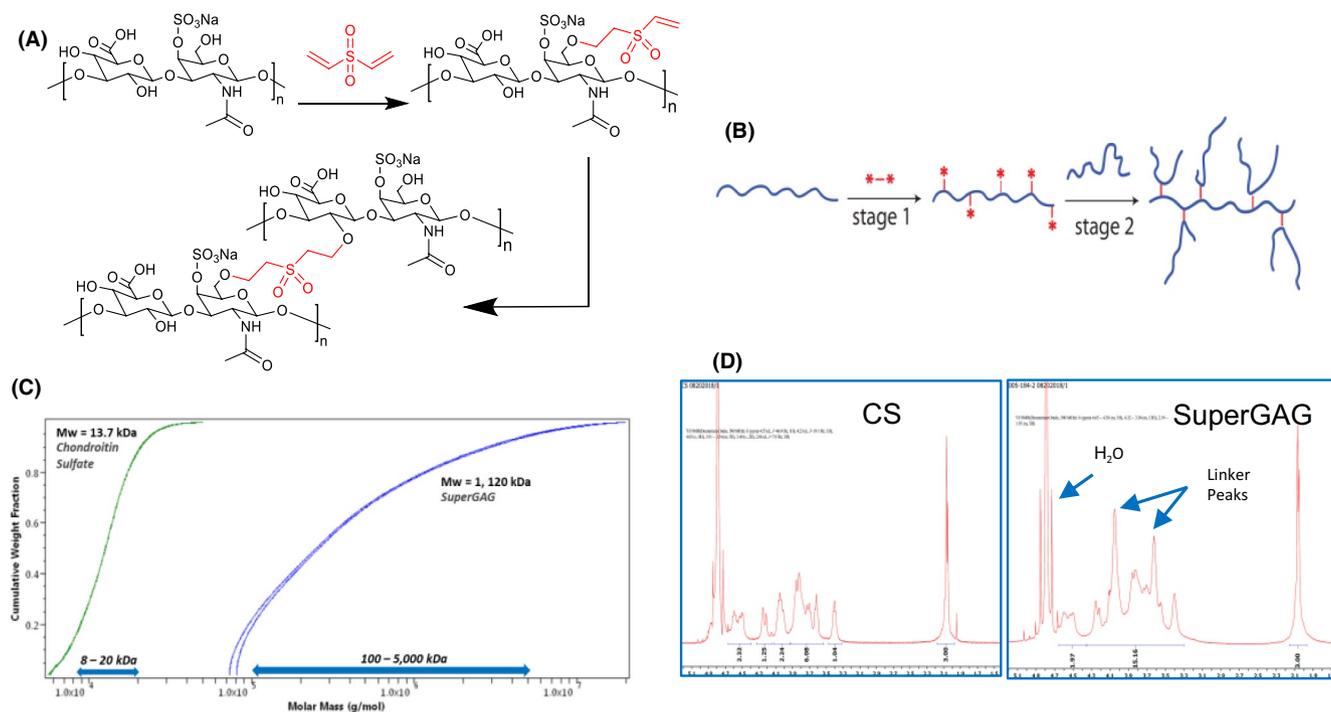


FIGURE 1 Synthesis of SuperGAG. (A) Overall synthetic schema using DVS to link CS chains. (B) Schematic for synthesis of branched, PG-like SuperGAG. (C) Molecular weight distributions of CS raw material vs. SuperGAG. The SuperGAG is broadly heterogeneous with >16 CS chains per SuperGAG molecule. (D) Proton NMR spectra measured in D$_2$O comparing SuperGAG with CS

·p<0.05,***p<0.001 compared to Vehicle, Two-Way ANOVA w/ Bonferroni's multi-comparison post-test.



**p<0.01,***p<0.001 compared to Vehicle, +p<0.05 Compared to CS, #p<0.05 Compared to Sham. Two-Way ANOVA w/ Bonferroni's multi-comparison post-test.

**FIGURE 2** SuperGAG instillates were more effective than vehicle control in restoring bladder impermeability in the protamine sulfate rat model. (A) TEER measurements of excised bladder membrane from the protamine sulfate-rat model performed 3 hours after treatment with biopolymers. (# of rats per group in parentheses). Sham treated controls showed TEER =2500 ± 249 $\Omega cm^{2}$.[42] (B) Restoration of impermeability reduces pain response. OVX female rats were infused intravesically with 1 mg/ml protamine sulfate, and 24 hr later treated with vehicle, CS (20 mg/ml) or SuperGAG-1 (20 mg/ml). Controls consisted of animals receiving a sham instillation instead of protamine sulfate or any treatment or protamine sulfate followed by vehicle. Bladder sensitivity was assessed 3 hours later using von Frey filaments applied to the suprapubic region. Each filament was applied for 1–2 sec for 10 applications. The filaments were tested from lowest to highest force. Sharp retraction of the abdomen, immediate licking or grooming or jumping was considered a positive response. At moderate pressures (2–4 g), SuperGAG reduced the pain response by half or more and was more effective than CS in relieving pain. At higher forces (15 g), the pressure likely affected organs other than the bladder and overwhelmed any palliative effect

highest pressures, which could be due to impinging on other organs. SuperGAG was more effective in reducing pain than CS at the same concentration.
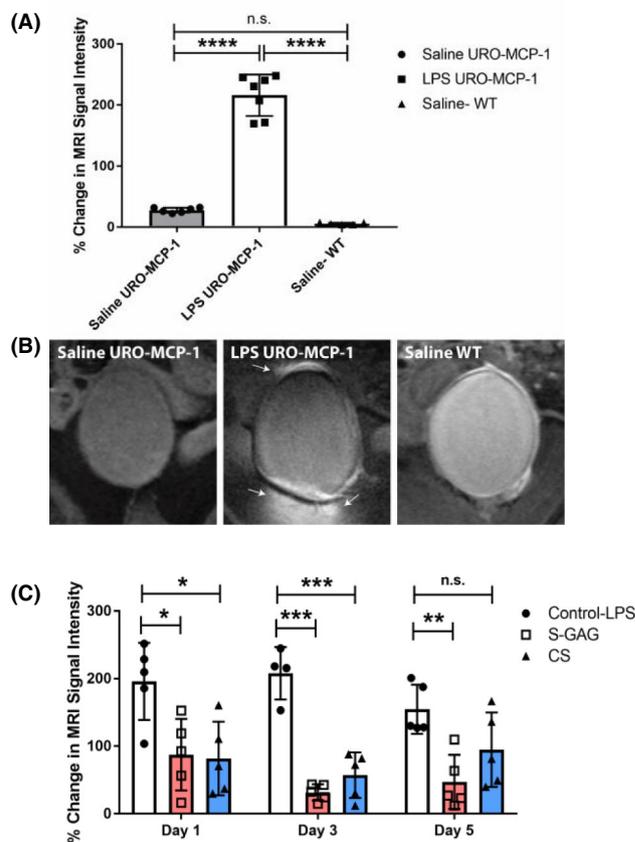






**FIGURE 3** Increased bladder permeability is present in the URO-MCP-1 IC mouse model. (A) Percent change in MRI signal intensities outside the bladder wall after intravesical injection of MRI contrast agent, Gd-DTPA, into the bladders of saline- (closed circles and dark grey bar; n = 6), LPS-treated (closed squares and white bar; n = 7) URO-MCP-1 mice, or saline-treated wild type (WT) mice (closed triangles and light grey bar; n = 5). There was a significant increase in the percent change in MRI signal intensity in LPS-treated URO-MCP-1 mice, compared to saline-treated URO-MCP-1 mice (****$p < 0.0001$), or the LPS-treated URO-MCP-1 mice, compared to saline-treated WT mice (****$p < 0.0001$). (B) Representative contrast-enhanced MR images of either saline- or LPS-treated URO-MCP-1 mouse bladders, and a saline-treated WT mouse bladder. Note hyper-intense regions of the bladder in the LPS-treated mouse bladder (white arrows). (C) SuperGAG restores increased bladder permeability to near-normal levels in a URO-MCP-1 model for interstitial cystitis. Percent change in MRI signal intensities outside the bladder wall after intravesical injection of MRI contrast agent, Gd-DTPA, into the bladders of LPS- (closed circles and white bar; n = 5), SuperGAG administered LPS-treated (open squares and red bar; n = 5), or chondroitin sulfate (CS)-treated (closed triangles and blue bar; n = 5) URO-MCP-1 mice. Equal weights of CS were instilled with the SuperGAG and the CS monomers (20 mg/ml), which is also the dose used clinically. There was a significant decrease in the percent change in MRI signal intensity (SI) in SuperGAG- or CS-administered LPS-treated URO-MCP-1 mice, compared to LPS-treated URO-MCP-1 mice (*$p < 0.05$ for both on day 1; ***$p < 0.0001$ for both on day 3). Only SuperGAG LPS URO-MCP-1 mice had significantly decreased the % change in MRI SI on day 5, compared to LPS URO-MCP-1 mice (**$p < 0.01$)

Figure 3 demonstrates that with the URO-MCP1 transgenic mouse model, and using CE-MRI to assess permeability, SuperGAG restores the bladder permeability to normal values. Figure 3A illustrates that the sub-noxious dose of LPS induces bladder permeability whereas instillation of saline does not, for either saline-treated URO-MCP1 or wild type mice. Figure 3B shows representative images of bladders, control, saline-treated URO-MCP1, protamine sulfate-treated and saline treated wild type mice. Note that in the rat protamine sulfate model normal control values are high (2500 $\Omega cm^2$) whereas in the mouse LPS model permeability is assessed directly with CE-MRI, and normal values are low. In the case of the mouse model, the control refers to values obtained with treatment to induce permeability, and then vehicle is administered instead of an agent to restore impermeability. Figure 3C shows the change over time determined by repeat determinations on days 1, 3, and 5 with the same mice treated either with LPS only (Control-PPS), SuperGAG (S-GAG) or CS. The small effect size in comparing the SuperGAG prep and CS would require a much larger sample size to test for statistical significance. Nonetheless, the data indicate that SuperGAG is more effective than CS, particularly at day 5 following treatment, as it was significantly lower than the control, whereas CS was not significantly different to control.

## 4 | DISCUSSION

The therapeutic options for IC/PBS patients are limited in effectiveness.[2,13] There is growing awareness that increased bladder permeability plays a central role in the pathophysiology of IC/BPS,[14,15] although it is by no means the sole contributor to the disease.[16-24] GAG replenishment to restore normal impermeability is among the more effective therapies with efficacy around 50% of patients.[29] Several reasons for limited effectiveness can be proposed. The disease could be progressive, and once it progresses to a chronic pain syndrome, the physical stimuli from penetration of urinary solutes into the bladder may not be needed to induce pain. Also, the administration schedules for intravesical GAG have not been optimized because the moderate response rate makes such studies difficult. Finally, currently available linear GAG biopolymers such as CS or hyaluronan do not offer the same dense, three-dimensional structure provided by the normal GAG layer on the luminal surface.

The SuperGAG described in the present study suggests an improved therapeutic approach that could increase the effectiveness of GAG replacement therapy for IC/BPS. Figure 4 illustrates a vicious cycle wherein loss of impermeability of the urothelium leads to inflammation and pain over the short run and in the long run to sustained inflammation that induces afferent and CNS remodeling leading to chronic, unremitting abdominal pain [31-35] that may not require the stimulus of urinary solutes (particularly $K^+$) to produce pain. Our approach is based on the premise that branched, highly reticulated, high molecular weight sulfated GAG biopolymers that mimic the three-dimensional structure of the natural proteoglycan-based GAG layer should be more effective in providing an ionic barrier to urinary
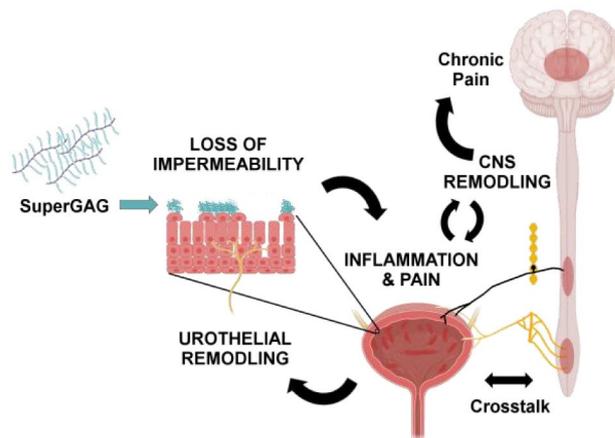


**FIGURE 4** SuperGAG and the progressive vicious cycle of IC/BPS. The loss of impermeability leads to pain as urinary solutes (K+) penetrate the urothelium leading to depolarization of sensory afferent bladder nerve up-regulation (parasympathetic pelvic nerves in yellow, sympathetic nerves in black) and other urinary solutes lead to inflammation with remodeling of the urothelium into a permeable state with focal loss of the umbrella cells and their attached GAG layer. At this stage, the symptoms vary in severity with the composition of the urine. Blocking permeability with SuperGAG (blue arrow) can ameliorate symptoms. It is possible also that partial regression of the urothelial permeability will occur. However, the disease is progressive, and in the longer-term afferent remodeling leads to unremitting pain that in turn leads to CNS remodeling. Organ crosstalk leads to a generalized, self-sustaining abdominal chronic pain syndrome (with sensitization or nerves in the brain and/or spinal cord – red ovals) such that the stimulus of urinary solutes is no longer required to produce pain. SuperGAG restores urothelial impermeability that over the long term may facilitate the eventual restoration of a normal urothelium and down-regulation of afferent and CNS remodeling

solutes than the currently available generic GAG biopolymers used therapeutically. Figure 1 illustrates the synthetic schema and demonstrates the SuperGAG can be prepared reproducibly. We clearly demonstrate in Figure 2 that the SuperGAG restores normal impermeability and also diminishes the abdominal pain response to induced permeability. Figure 3 shows that the effect of restoring impermeability is not limited to the rat model but occurs in the currently best accepted animal model of IC/BPS. The SuperGAG offers improved duration of action; although the effect of SuperGAG was waning at 5 days, it was nonetheless statistically significant from the control whereas the effect of CS was not. One of the limitations of these studies is that in both models the urothelium normally recovers in 5–7 days. These findings suggest further improvement in SuperGAG efficacy could achieve a treatment with an acceptable treating interval that continuously maintains bladder impermeability.

Figure 4 illustrates how SuperGAG biopolymers could break the vicious cycle of IC/BPS by effectively restoring bladder impermeability. Without the continuous noxious stimulation of urinary solutes, it is possible that over a long term the bladder could return to normal. Second, the use of MRI to monitor bladder permeability provides a means to optimize the administration schedule so that impermeability

is maintained between doses. We propose that maintaining continuous impermeability is crucial to preventing inflammation and allowing the normal, impermeable urothelium to be restored and potentially achieving downregulation of pain receptors and possibly the CNS changes that accompany chronic pain. In any case, without such long-term maintenance of impermeability, healing is very unlikely.

We consider the current communication to be a demonstration of feasibility for developing a more proteoglycan-like therapeutic for IC/BPS. The preliminary data presented here indicate an improvement in efficacy over CS itself. Planned future research to take this concept to the clinic will include further optimization of SuperGAG biopolymer compositions to enhance binding to the urothelium to enhance duration of action, additional animal studies to assess improved efficacy and optimize dosing frequency to maintain impermeability in animals. Such studies could be performed using MRI and a Gd-labeled SuperGAG.[47,48] First in human clinical trials will then assess optimal dosing frequency in humans using well validated patient-reported outcomes measures (e.g., O'Leary-Sant questionnaires) [49] and bladder MRI permeability measures that can determine for how long SuperGAG provides protection.

We conclude that high molecular weight SuperGAG biopolymers are effective in restoring urothelial impermeability and reducing pain produced by loss of the GAG layer on the urothelium in rodent models of bladder permeability. SuperGAG biopolymers could offer a novel and effective therapy for IC/BPS, particularly if combined with MRI to assess the efficacy of the therapy.

## CONFLICTS OF INTEREST

REH, HCS, and THJ are employed by Glycologix, LLC and GRS is a member of the Scientific Advisory Board of Glycologix, LLC.

## FUNDING INFORMATION

## DATA AVAILABILITY STATEMENT

Data will be made available to qualified investigators on request to the corresponding author.

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