Author's response to reviews

Title: Exogenous Glycosaminoglycans Coat Damaged Bladder Surfaces in Experimentally Damaged Mouse Bladder

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The authors wish to express their thanks for the collegial and helpful reviews by Dr Lewis (Reviewer 1) and Dr. Whitmore (Reviewer 2).

Reviewer 1.

The only way to obtain a corresponding light micrograph would have been to have stained the section used for confocal microscopy after obtaining the fluorescent image. We apologize for not thinking of this originally, but we have examined so many bladder sections labeled this way that we can easily see the urothelium. We failed to recognize that few readers are likely to have our experience in viewing such sections. Cutting serial sections and staining a section adjacent to the section examined for fluorescence really isn't an option because obtaining serial sections from frozen material that yield corresponding histology is difficult, at best. Sections tend to shrivel or lose their shape, so that serial frozen sections lose their comparability. As an alternative to repeating the experiment, we have marked the urothelium of the protamine-treated bladder with arrows. In addition, we have added both H&E and Alcian Blue stained images to better demonstrate the nature of the damage produced by each model.

The level of binding is quantified in Table 1. The sections shown are representative. Three mice per treatment were examined, and at least two sections were examined per mouse.

Reviewer 2.

The number of mice per treatment (3), the number of sections (2) and the fact that the sections were examined without knowledge of the investigator is now so stated.

We did not provide references for the bladder damage models because they have appeared in the literature in a number of variations, depending upon the author, the species (mouse, rabbit, mouse). Thus, the choice of a reference would be rather arbitrary. We would, however, be glad to attempt to select a reference for each if the reviewer feels it is really necessary. However, in response to both reviewers' comments, we included additional photomicrographs documenting the results of each model, which will, perhaps, serve to document each of these models for the mouse.

The concentration of protamine was 1 mg/ml.

For this reviewer's information, our speculation that protease exclusion might be involved with symptom relief is based on an earlier finding by Dr. Lewis that urine contains proteases that can degrade ion channels. We speculated in reference 20 that the function of the "GAG Layer" may be to exclude proteases, since both his experimental evidence in Ussing chambers and my calculations based on actual measurements of the amount of GAG at the bladder surface suggest that the "GAG Layer" does not provide the primary permeability barrier. We hesitate to speculate overly, but at this reviewer's request have added a clearly labeled speculative statement that also includes the earlier reference to Dr. Lewis' findings.