

Novel Biomimetic Aggrecan for Treatment of Urinary Incontinence.

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Statement of Purpose: Stress urinary incontinence (SUI) plagues 56% of post-menopausal women, and 11% women after age 65 have surgery for SUI (1). SUI is associated with stiffening and decreased volume of the urethra making it more difficult for the urethra to close. Current treatments, such as narrowing the urethra lumen with bulking agents, are successful for an immediate elimination of incontinence, however are limited in efficacy in that they last only six months due to migration of the bulking materials from the urethra (2). We propose a new strategy where a novel biomimetic aggrecan (BA) can be injected into the urethra to stop stress incontinence by increasing tissue volume, reducing tissue stiffness and importantly remaining in the urethra due to interaction between the BA with urethral matrix collagen fibrils. Our recently developed biomimetic aggrecan mimics three dimensional bottle-brush architecture and osmotic pressure of natural aggrecan (3,4).

Here, we report our initial results on molecular engineering of urethra tissue (porcine, cadaver model) with biomimetic aggrecan.

Methods: Biomimetic aggrecan synthesized using a “grafting to” strategy, in which linear chondroitin sulfate (CS) molecules (Sigma-Aldrich) with primary amine end groups were covalently coupled to functional groups along enzymatically-resistant polymer backbone poly(acrylic acid) (PAA) (MW 250kDa, Sigma-Aldrich). Bottle brush configuration was confirmed with AFM (image obtained in Dr Ortiz lab, MIT). Cytocompatibility of our biomimetic molecule was assessed with L929 fibroblasts. Osmotic pressure and water uptake of the BA solution were measured via gel osmometry (Sephadex G-50) and TGA, respectively.

Porcine urethra samples were obtained from Animal Technologies, Inc. 1 ml total volume of BA solution (1X PBS, 50 mg/ml) was injected into three sites of the perimeter of the urethral opening, according to the current injection protocol for clinical injection of bulking agents. Changes in tissue stiffness and volume were assessed with mechanical tensile testing and Micro-CT, respectively. To assess integration of BA with the urethra tissue, BA molecules were additionally fluorescent-tagged with DCCH (Invitrogen); injected urethra samples were equilibrated in a fluid bath for 24 hrs and imaged via confocal microscopy.

Results:

CS molecules were successfully incorporated onto a polymer backbone, and AFM image of BA sample showed bottle brush structure with a size of a molecule of at least 160 nm (Fig 1). This compares to ~100-300 nm for natural aggrecan. BA was shown to be cytocompatible.

BA solution had a statistically higher osmotic pressure than CS alone ($p < 0.001$ at 50mg/ml) through the range of

physiologically relevant concentrations and was in the range of osmotic pressure of natural aggrecan.

From water uptake studies, CS and natural aggrecan were shown to have comparable water uptake at ~40%, while BA demonstrated ~60% uptake.

Tensile testing results indicated that urethra stiffness was reduced by ~49% as a result of BA injection. Urethra volume was increased by 23%, as determined with Micro-CT (Fig. 2 (left)). These results imply that the tissue had a higher osmotic pressure after the injection retaining more water. As indicated by fluorescence imaging results, BA molecules remained in a urethra sample after 24 hrs of equilibration and integrated well with the tissue (Fig. 2 (right)).

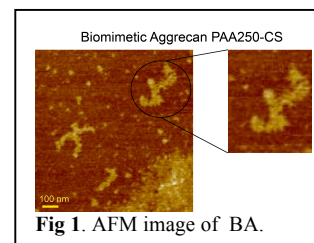


Fig 1. AFM image of BA.

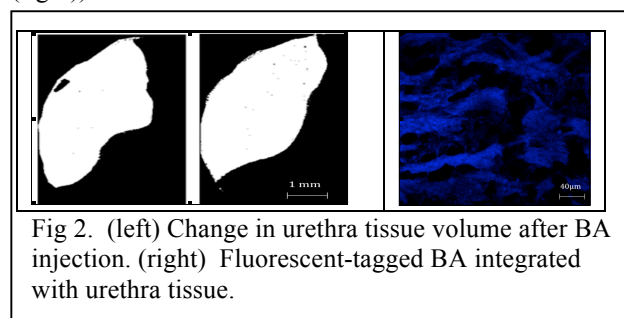


Fig 2. (left) Change in urethra tissue volume after BA injection. (right) Fluorescent-tagged BA integrated with urethra tissue.

Conclusions:

Recently developed biomimetic aggrecan that mimics the structure and physical properties of the natural molecule can be potentially used for treatment of urinary incontinence. Reduction in stiffness and increase in volume observed in our initial experiments are promising to have the ability to reverse the deteriorative changes that have been shown in incontinent women.

References:

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