222

Sadeghi Z[1], Wang N[2], Kavran M[1], Daneshgari F[1], Recum H V[2], Hijaz A[1]
1. *Urology Institute, University Hospitals Case Medical Center; Department of Urology, Case Western Reserve University School of Medicine, Cleveland, OH;,* ***2.*** *Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH;*

FEASIBILITY OF INTRAVAGINAL IMPLANTED AFFINITY-BASED CCL7 SLOW RELEASING HYDROGELS FOR STIMULATING STEM CELL MIGRATION IN STRESS URINARY INCONTINENCE

Hypothesis / aims of study

Chemokine (C-C motif) ligand 7 (CCL7, Previously called MCP3) is significantly over expressed in rat urethral and vaginal tissues immediately following induction of stress urinary incontinence in a rat model simulating birth trauma.[1] CCL7 over expression has shown potency for stimulating targeted stem cell migration. [2] “Affinity-based” delivery is the controlled loading and release of therapeutics using molecular interactions to obtain the increased loading and prolonged release not available by diffusion alone. Our aim in this project is to prepare affinity-based hydrogels that slow release CCL7, applicable for local implantation in periurethral area which could provide therapeutic levels of CCL7 on the order of weeks or longer to stimulate host stem cell migration.

Study design, materials and methods

Hydrogel Synthesis and Washing: Hydrogels are a network of crosslinked hydrophilic polymers commonly used in drug delivery. Hydrogels used in this study were synthesized by crosslinking bovine serum albumin (BSA) with heparin using EDC chemistry. Binding interactions between heparin and CCL7 using surface Plasmon resonance analysis (data not show) showed strong affinity between heparin and CCL7. BSA was chosen as a base relatively inert material with readily available amines for crosslinking chemistry. Mixture of totaling 100mg of 25:75 and 100:0 heparin to BSA ratio (ratio chosen based on previous release studies with other cytokines) was weighed and dissolved in 500ul of 0.1M 2-(*N*-morpholino) ethanesulfonic acid (MES) solution (pH adjusted to 5.2 using 0.1N NaOH). Separately, 40ul solutions of 1mg/ml of EDC in 0.1M MES were prepared. On ice, each 500ul heparin/BSA solution was thoroughly mixed and vortexed with the 40ul EDC crosslinker solution in a 15mm scintillation vial. The gels were slowly brought to room temperature and left to cure overnight to form the hydrogels. After 24 hrs, the gels were removed from the vials and washed 3 times. Each wash cycle consisted of swelling the gels in 10ml of PBS and leaving them in gentle agitation for 1 hour. At the end of the 3 wash cycles, the gels were left in gentle agitation overnight. After the wash, 3mm circular drug release gels were made using a punch pin. The gels were dried overnight before drug loading.
Drug Loading of Hydrogels: CCL7 was loaded into the hydrogels by hydrodynamic loading. Dried gels were swelled in 200ug/ml CCL7 solutions (in PBS) in wells of a 24 well plate. The wells were sufficiently large to accommodate the size of the fully swollen gel. The gels were allowed to swell for 48hrs to allow the gels to fully load. The fully loaded gels were weighed before start of the release study.
In Vitro Drug Release: CCL7 release profiles from the hydrogels were characterized by an in vitro release study. The release study was carried out by incubating each loaded gel into 1mL of 0.1% BSA in PBS solution in a 1.5 cm diameter well. The gels were incubated at 37^{o}C under gentle agitation. At each time point, each gel was removed from its release solution and was subsequently placed into a new well containing fresh release buffer. The buffer containing already released aliquots was stored in 500ul aliquots at -20^{o}C for later analysis. Daily samples were taken for the first 2 weeks, followed by sampling every other day for 4 weeks. Levels of 5P12-RANTES in each aliquot were measured by CCL7 ELISA. Release profiles showing daily release were plotted against days of release to determine the total release profile.
Gel implantation: To evaluate the anatomical feasibility of gel implantation, in a female SD rat, 2 affinity based Heparin/BSA CCL7 releasing gels were implanted sub-epithelially in the anterior wall of vagina alongside the urethra. Three weeks later the vagina and urethra was dissected out of the rat body. The vagina was cut longitudinally from the posterior wall and the interior side of the vagina lumen was exposed to look for the implanted gel.

Results

As shown in the Figure 1, CCL7 released at each time point from hydrogels. Heparin/BSA (♦) gels showed a decrease in burst release in the early time points (first 2 days) and a more sustained released over time (weeks) (affinity based release). BSA only gels (■) gels showed a rapid initial burst release (first 2 days) and much lower levels of release over time (weeks).

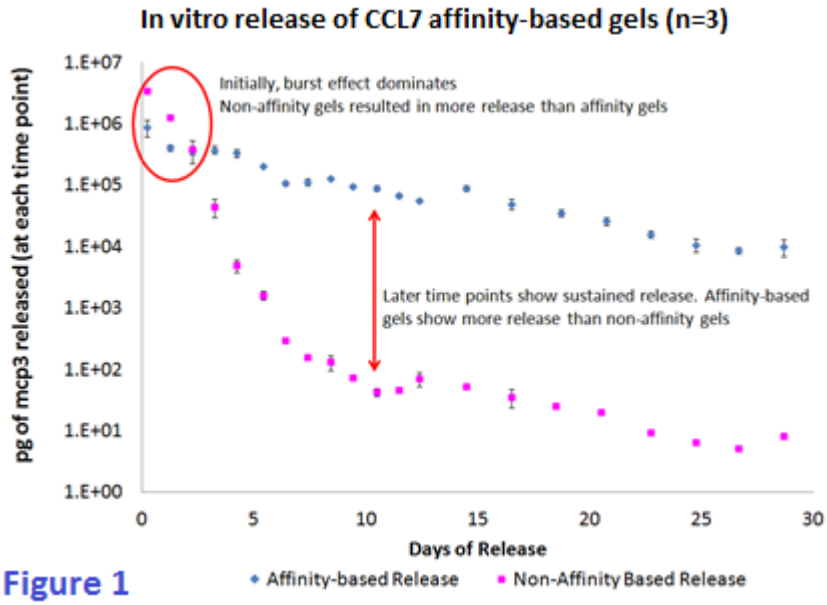

Figure 1

We successfully identified the CCL7 affinity-based gels 3 weeks post-implantation, alongside the urethra at the site of implantation. No tissue disruption, herniation or scar was visible in the vaginal wall. (Figure 2)

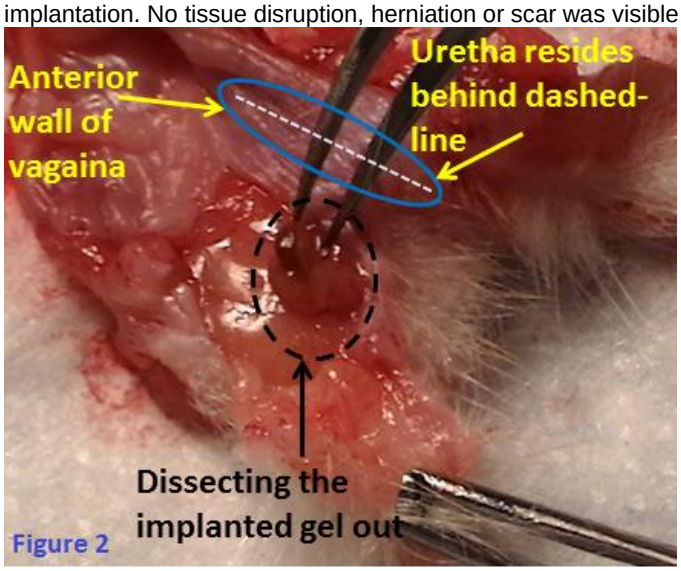

Figure 2

Interpretation of results

Results suggest an affinity-based system with conjugated heparin may be used for sustained release of CCL7. Affinity based gels demonstrated feasible embedding and durable compatibility and stability after implantation in vaginal wall.

Concluding message

Successful characterization and control of such a repair mechanism in the lower urinary tract would introduce the potential for novel non-operative treatments and/or preventive measures for stress urinary incontinence.

References

1. Wood, H.M., M. Kuang, L. Woo, et al. Cytokine expression after vaginal distention of different durations in virgin Sprague-Dawley rats. THE JOURNAL OF UROLOGY 2008; 180(2): 753-759.
2. Schenk, S., N. Mal, A. Finan, et al. Monocyte chemotactic protein-3 is a myocardial mesenchymal stem cell homing factor. STEM CELLS 2007; 25(1): 245-251.

Disclosures

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