

## Immunotherapy with Shear-thinning Injectable Hydrogels to Treat Obstructive Nephropathy

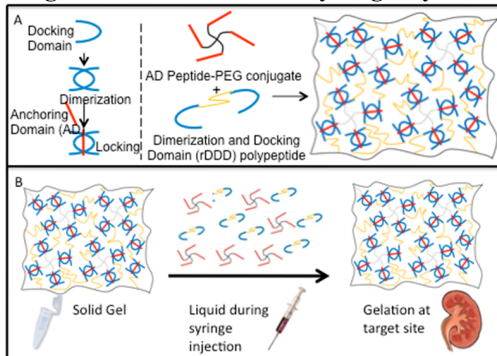
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**Statement of Purpose:** Congenital obstructive nephropathy is the leading cause of chronic kidney disease (CKD) in children. Despite early surgical repair, upwards of 50% of affected children progress to end-stage kidney disease. The cellular mechanisms of kidney injury caused by obstructive nephropathy are interstitial inflammation, fibrosis, and apoptosis. Rodent models can be used to simulate obstructive nephropathy in the human kidney. Here, we developed shear-thinning hydrogels for the local delivery of IL-10 to abate the progression of inflammation and fibrosis that leads to CKD.

**Methods: Injectable Hydrogels:** Injectable Dock-n-Lock gels were developed as previously reported<sup>1</sup> (Fig. 1) and utilize the docking and dimerization domain of c-AMP-dependent protein kinase A with the anchoring domain (AD) of A-kinase anchoring proteins. The AD is conjugated to the ends of four-arm polyethylene glycol (4aPEG). IL-10 was added to the gel or phosphate buffered saline solution at a concentration of 0.33 µg/µL. **Optical Imaging:** The fluorescent marker Cy 5.5 was covalently bonded to the AD-peptide to allow for *in vivo* optical imaging and quantification of gel degradation (LICOR), (Fig. 2, A). **Study Design:** Eight cohorts were studied (7, 21, 35 days, n=4): healthy, sham operation, healthy injected with MSA, healthy + gel, unilateral ureteral obstruction (UUO), UUO + IL-10, UUO + gel, UUO + gel/IL-10. 15 µL of IL-10 solution, gel, or gel/IL-10 was injected into the left kidney via retroperitoneal approach 3 days after the initial ureteral obstruction or sham operation. **Histology:** Immunohistochemistry (IHC) was performed on paraffin sections to identify macrophages and apoptotic cells via rat anti-CD68 IgG (abcam) and Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) (Invitrogen), respectively, and trichrome stain was used to evaluate fibrosis. Cells and total area were quantified (20 images/section) for IHC and total fibrotic area was quantified for trichrome. ANOVA with Tukey posthoc was used to determine significance.

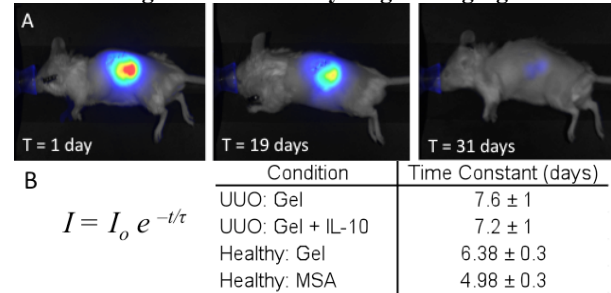
**Figure 1. Dock and Lock Hydrogel System**



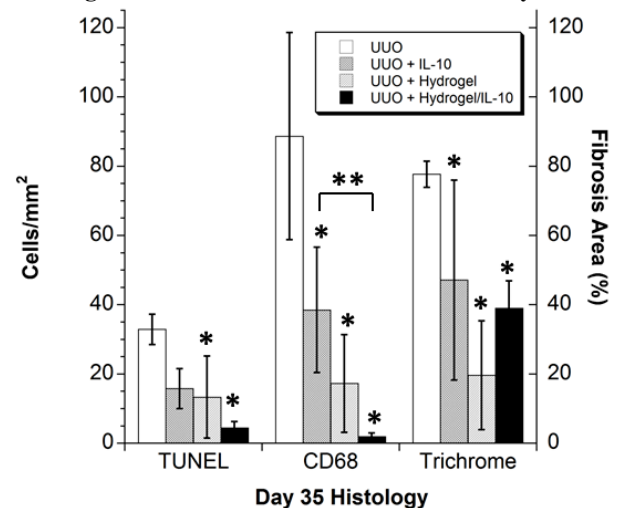
**Results:** MSA clears faster than 4aPEG gel when injected into healthy kidneys. Gels with and without IL-10 have similar erosion rates when injected into obstructed kidneys (Fig. 2B). In the control groups (healthy, sham,

healthy + MSA, healthy + gel), there was no significant difference in macrophage infiltration, apoptosis or fibrosis. In comparing the treatment groups (UUO + IL-10, UUO + gel, UUO + gel/IL-10) to the untreated UUO, macrophage infiltration and apoptosis were significantly reduced at day 21 and 35. By day 35, adding the IL-10 via gel injection reduced macrophage infiltration more than IL-10 alone and IL-10 alone did not reduce apoptosis. Fibrosis was decreased by day 35 in all three treatment groups (Fig. 3).

**Figure 2. In Vivo Hydrogel Imaging**



**Figure 3. Quantification of Markers at Day 35**



**Conclusions:** Shear-thinning hydrogels were synthesized that permit facile local delivery of immunotherapy to both healthy and obstructed kidneys, as well as *in vivo* optical imaging via the covalent attachment of a far-infrared marker to the hydrogel. Injecting the gel into healthy kidney did not induce inflammation or scarring, and all quantified markers were not statistically different than untreated or sham controls. Renal inflammation and scarring was reduced in an animal model of CKD by using an injectable hydrogel for delivery of IL-10, a potent anti-inflammatory cytokine. Injectable hydrogels are a novel and clinically viable option to treat CKD.

**References:** 1. H.D. Lu, M.B. Charati, I.L. Kim, J.A. Burdick. *Biomaterials*. 33:2145-2153,2012.