# Urinary Bladder Regeneration Utilizing Bone Marrow Derived Mesenchymal Stem Cell Seeded Elastomeric poly(1,8-octanediol-co-citrate) Based Thin Films

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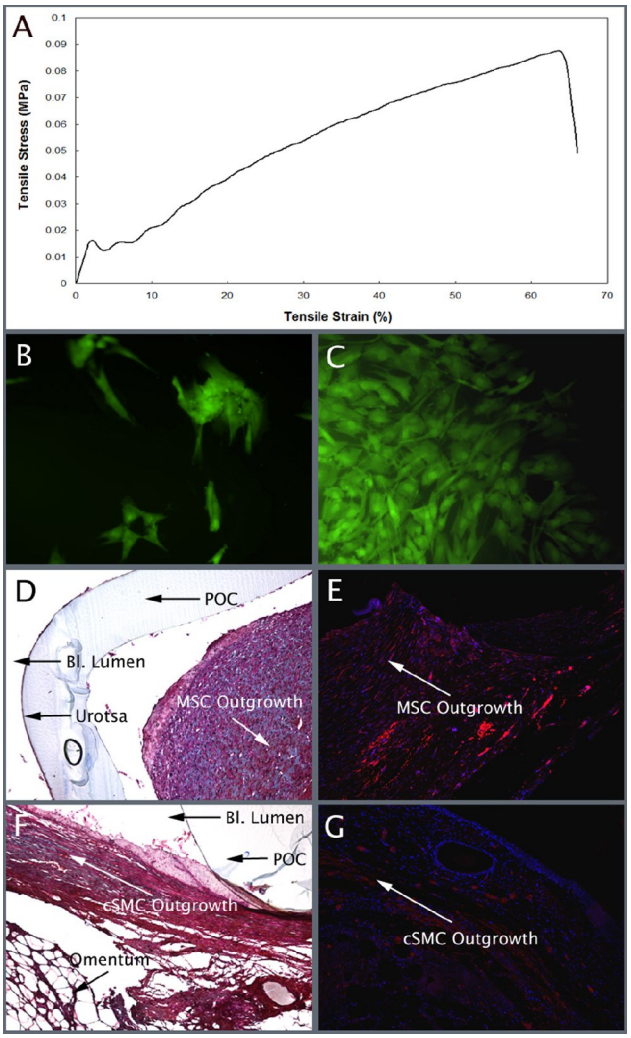
**Statement of Purpose:** Acquired or developmental disorders affecting the function of the urinary bladder can lead to a myriad of pathological conditions. Clinical studies with regards to bladder regeneration have yielded inconclusive results[1] due to the possible use of diseased cell types and primitive scaffold design suggesting that alternative cell and scaffold sources should be examined. Multipotent bone marrow (BM) derived mesenchymal stem cells (MSCs) represent highly characterized cells capable of terminal transdifferentiation into multiple cell lineages[2] and thus may be suitable for bladder regeneration studies when combined with an appropriate synthetic scaffold. Poly(1,8-octanediol-co-citrate) (POC) is an elastomeric compound that has been demonstrated to support the growth of a variety of cell types and can be synthesized in a reproducible, controlled manner to specific mechanical properties.[3] The chemical preparation of POC allows for the synthesis of a biodegradable, non-toxic thin film that can be seeded to high cell densities and act as a sutural material *in vivo*. In this study, we demonstrate the construction of POC based thin films (POCf) and its functional utility within an *in vivo* rat bladder augmentation model.

**Methods**: Equimolar amounts of citric acid and 1,8 octanediol were combined and melted at 160°C. Gradual cooling to 140°C for 25 min was performed to create POC pre-polymer. Pre-polymer was post-polymerized in a flat bottomed glass dish for 7 days at 55°C, removed from its mold and washed in media for 24 hrs with changes every 6 hrs. Tensile mechanical tests were performed with a mechanical tester equipped with a 500N load cell. 5.0cm x 1.0cm POCfs were pulled at 500mm/min and Young's modulus was determined. Human BM MSCs/Urotsa (an immortalized urothelial cell line), human mcherry labeled bladder smooth muscle cells (cSMCs)/Urotsa were seeded on opposing sides of POCfs (0.50cm x 0.75cm) at 15K cells/cm$^2$ and cultured for 1 week. Cell viability was determined *in vitro* up to 21 days post-seeding via a Calcein Live/Dead Staining Assay (Molecular Probes). A 30% bladder defect was created via partial cystectomy in a nude rat bladder and replaced with the aforementioned cell seeded POCfs or unseeded POCfs enveloped with an omental wrap. Rats were sacrificed 4 weeks post implantation and augmented bladders were paraffin embedded and subjected to Masson's Trichrome and anti human γ-tubulin (1:100, SCBT) staining.

**Results:** Synthesized POCfs revealed a Young's modulus of 138.53kPa ± 2.85kPa as calculated by the slope of the tensile stress/strain data (Fig A). POCfs were able to elongate to 137% of their initial length without permanent deformation demonstrating their high elastic potential. *in vitro* MSC viability at day 1 (Fig B) and day 21 (Fig C) post seeding was >98% on POCfs as quantitatively determined by live/dead staining. Trichrome staining of rat bladders augmented with an MSC/Urotsa seeded POCf (Fig D) grew to thickness levels greater than a comparably seeded cSMC/Urotsa POCf (Fig F). Unseeded POCf animal controls died 1 day post surgery. Collagen to muscle ratio was also comparable between MSC and SMC groups. γ-tubulin staining of the MSC/Urotsa POCfs indicated that cell outgrowth was of human origin (Fig E) as also seen with directly labeled cSMCs (Fig G).




**Conclusions:** Data demonstrate that POCfs can be synthesized in a controlled manner to create elastomeric thin films of specific moduli. POCfs sustain MSCs at high viability *in vitro* and also help support the regeneration of bladder tissue *in vivo.* The combinatorial effects of MSC seeded POCfs may be beneficial for bladder regeneration.

**References:**

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