Novel Biodegradable, Elastic Polymer enabled Biomimetic Urinary Bladder

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Statement of Purpose: Urinary bladder dysfunction is a common chronic problem and may require a bladder reconstruction surgery to restore its normal function. Total number of patients awaiting bladder transplants is estimated to be about 57,200 per year in U.S.[1] Urinary bladder is a contractile organ which acts as a reservoir for temporary storage of urine. Development of urinary bladder is a long and complex biological process: a series of events like change in size, capacity, muscle functions, SMC and ECM development that occur during bladder development contribute for the normal functioning of the bladder. In our present study we have designed a novel approach using an elastic, biodegradable polymeric bladder pouch built in a bioreactor system to mimic the normal bladder development process, continuous filling and voiding behavior of bladder.

Methods: Synthesis and characterization of a novel biodegradable, elastic polymer (CUPE): Poly (octanediol) (POC) prepolymer was prepared by polycondensation reaction using 1, 8-octanediol and citric acid as monomers at a molar ratio of 1.1:1. After purification, the prepolymer was dissolved in 1, 4-dioxane to make 3% wt solution. Hexamethyl di-isocyanate (HDI) was added to the solution at a ratio of 1:0.9 in proportion to the molecular weight of the pre-POC. Stannous octoate was used as catalyst (0.1% wt). The reaction was run at 55ºC with a Fourier transformed infrared (FT-IR) check regularly and terminated when the isocyanate peak disappeared in the FT-IR spectrum. Films of pre-CUPE were made by solvent evaporation method followed by a postpolymerization step at 80°C to obtain crosslinked urethane-doped polyester (CUPE). These films were tested for the mechanical properties using MTS Insight 2 mechanical tester loaded with a 500N load cell.[2]

Bladder derived smooth muscle cell culture: Primary smooth muscle cells were dissected from the bladder wall of neonatal rats (P7-10). After scraping urothelium, the bladder tissue was minced and enzymatically digested. The dissociated smooth muscle cells were cultured in DMEM/F12 medium containing 10% fetal bovine serum.

Smooth muscle cell growth on CUPE polymer: The CUPE polymer was coated on glass coverslip. The bladder derived smooth muscle cells were then seeded on the coverslip and cultured for seven days. Glass coverslips without CUPE coating were used as a control. Seven days after, the cells were fixed and immunostained for smooth muscle actin.

Fabrication of a bioreactor to mimic bladder system *in vitro*: Hollow polymer pouches of CUPE were fabricated by dipping the derlin molds of elliptical shape (of 6cm length and 1cm diameter) in pre-polymer solution followed by the solvent evaporation and postpolymerization steps. These molds were de-molded to get a hollow pouch. These pouches were connected to inlet and outlet tubes using micro hose tube clamps and sealed well to avoid leakage. A bioreactor system was designed to enable the repeated filling and voiding of the polymeric pouch using a feedback circuit. The pouch was continuously filled using a syringe pump at a constant flowrate of 5ml/hr through the inlet tube and drained by a solenoid valve connected to the outlet tube. A regulatory circuit with two delay timer modules (ELK products Inc., NC, USA) was designed. The timer1 was configured to activate the timer2 for 30seconds by applying a trigger for every 30 minutes. This regulatory circuit was used to open the solenoid valve for 30 seconds for every 30 minutes. The efficiency of the system to fill and drain the CUPE pouch was evaluated using phosphate buffered saline (PBS).

Results: The results of FT-IR spectra showed no peak for isocyanate (at 2267cm^{-1}) indicating the incorporation of all isocyanate groups into the polymer chains and so the reaction was terminated. Mechanical testing of CUPE films reported the formation of a highly elastic polymer with a tensile strength of 32.10±2.69 (Mpa), Young's modulus of 5.84±1.84 (Mpa), and elongation percent of 278.24±10.12 making it suitable for the design. Hollow polymer pouches of uniform and thick polymer film were obtained after demolding [Figure 2]. The shape of the mold used aided for easy demolding of the pouch without any break.

Immunostaining of bladder derived smooth muscle cells (bSMCs) grown on the CUPE polymer demonstrated a robust growth with a positive staining to smooth muscle α-actin [Figure 1].

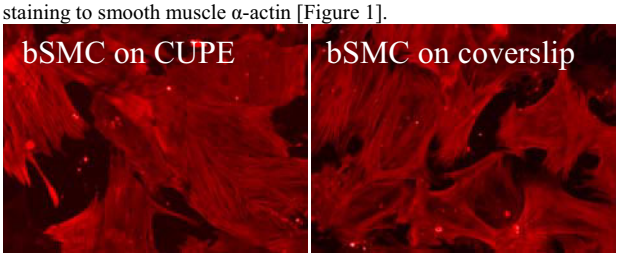

Figure 1. Immunostaining of bladder derived smooth muscle cells.

The bladder bioreactor system was successful in maintaining and regulating the continuous filling and voiding of CUPE pouch without any leakage. The regulatory circuit opened the valve at every 30 minutes and the valve remained open for 30 seconds draining half the volume of pouch. Inflation and deflation of the bladder pouch was observed by stretching and contracting the elastic pouch while filling and voiding respectively [Figure 3].

Figure 2. Derlin mold (A) used for fabrication of hollow polymer pouch (B).

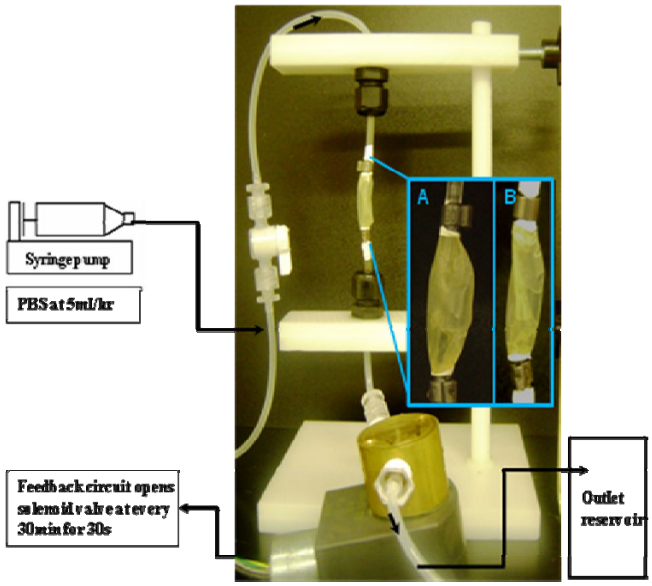

Figure 3. Repeated filling and voiding biomimetic bladder bioreactor with inflated (A) and deflated bladder (B) in the inlet.

Conclusions: We have devised a bioreactor model and successfully demonstrated the inflation and deflation of a novel elastic, biodegradable polymeric bladder while filling and voiding respectively, mimicking the natural bladder function.

References:

1. Ki Dong Park. Yonsei medical journal, vol. 41, NO.6, pp.780-788, 2000.
2. Jagannath Dey. Biomaterials 29 (2008) 4637–4649.