Sustained Intravesical Drug Delivery Using Thermosensitive Hydrogel

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Purpose. Direct instillation of drug solutions into the bladder through a urethral catheter (i.e., intravesical therapy) evades systemic adverse effects of drugs used for bladder diseases. However, conventional vehicles for these drugs fail to extend duration of drug exposure in the bladder beyond the first voiding of urine postinstillation. The current study seeks to overcome the aforementioned inherent limitation of intravesical drug administration by using thermosensitive hydrogel as a matrix for sustained intravesical drug delivery.

Methods. Under halothane anesthesia, normal adult female Sprague-Dawley rats were catheterized with PE-50 tubing to instill either 0.02% w/v solution of fluorescein isothiocyanate (FITC) or the same amount of FITC in a 30% w/v dispersion of thermosensitive {Poly-(ethylene glycol)-Poly[lactic acid-co-glycolic acid]-Poly(ethylene glycol)} (PEG-PLGA-PEG) polymer in a 0.1 M phosphate buffer. After instillations, rats were kept in metabolic cages for urine collection. Fluorescence emanating from FITC was measured in the urine at various time points up to 24 h after instillation. A rat model of cyclophosphamide-induced cystitis was chosen for the efficacy study using misoprostol as a model drug entrapped in the thermosensitive hydrogel in place of FITC. Efficacy of hydrogel containing misoprostol was compared against rat groups instilled with saline, hydrogel, and misoprostol independently.

Results. Prolonged drug exposure to the bladder afforded by hydrogel was evident from the time course of FITC elimination in the urine and by the green fluorescence of FITC seen at the bladder surface when isolated 24 h after instillation. Rats instilled with free FITC voided almost all of the fluorescence in the urine within the first 8 h, whereas rats instilled with hydrogel encapsulated FITC showed sustained release up to 24 h after instillation. Using a cyclophosphamide-induced cystitis model, rats instilled with misoprostol, a synthetic PGE1 analog, showed significantly reduced frequency of urine voiding ($p < 0.05$) as compared to the rats instilled with saline. Histological examination of the urothelium showed near normal morphology in rats instilled with misoprostol in hydrogel, whereas extensive tissue damage was observed in rats instilled with saline.

Conclusion. Our study showed that PEG-PLGA-PEG polymer could be used as a viable sustained drug delivery system for intravesical therapy of diseases of the bladder such as cystitis using misoprostol.

KEY WORDS: fluorescien isothiocyanate; hydrogel; misoprostol, urine.

INTRODUCTION

Intravesical therapy consists of drugs being placed directly into the bladder through a urethral catheter. Unlike systemic chemotherapy, responses to intravesical therapy are directly proportional to drug concentration rather than drug dose as shown for topical chemotherapy (1). However, duration of drug exposure so crucial for therapeutic response is usually limited by bladder capacity and short with the use of conventional vehicles in intravesical therapy (2). Ueda *et al.* showed that doxorubicin remained longer within the urinary bladder tissue of patients when administered together with a mucoadhesive agent hydroxypropylcellulose, and higher concentrations of drugs were achieved in tumorous tissue than in normal tissue (3).

Inadequate drug delivery by conventional vehicles justifies the search for new agents to overcome the limitations inherent in intravesical route of drug administration. We propose to use a thermosensitive polymer as a vehicle for extending drug exposure in the bladder beyond the voiding of urine postinstillation. The dispersion of this thermosensitive triblock co-polymer {Poly(ethylene glycol)-Poly[lactic acid-co-glycolic acid]-Poly(ethylene glycol)}(PEG-PLGA-PEG), in 0.1 M phosphate buffer exists in a sol state at room temperature or below but converts to a gel state at elevated temperature such as 37°C. Temperature-sensitive gelation of the instilled polymeric dispersion is expected to happen inside the bladder, as rat body temperature is 37°C. The hydrogel once formed inside the bladder can act as a matrix filled with drug for maintaining a prolonged exposure of drugs at the urothelium. Successful sustained intravesical drug delivery can eliminate multiple catheterizations leading to improved patient compliance, so important for the success of any form of therapy.

The thermosensitive and biodegradable characteristics of the triblock co-polymer selected for our study have widely been reported (4–7). As previously reported from our lab, a polymer of similar chemical composition was used as a matrix for a controlled gene delivery system (8). For the purpose of intravesical application, we modified the above polymer by increasing its hydrophobic content to improve the robustness of the formed hydrogel in the bladder to endure the urine constituents such as urea and electrolytes. Hydrogel used in the proposed study is capable of accomplishing the twin goals of semisolid consistency after it is inside the bladder and the ability to be injected through a PE-50 catheter.

The current study first used fluorescein isothiocyanate (FITC) as a model drug to demonstrate sustained intravesical drug delivery. Further validation of the concept was done in efficacy studies with misoprostol delivered using the hydrogel. Misoprostol is widely used in the clinic for the treatment of gastric and duodenal ulcer induced by chronic consumption of NSAIDs (nonsteroidal anti-inflammatory drugs) such as aspirin and ibuoprofen. Misoprostol is a stable analog of PGE1, a cytoprotective eicosanoid (cyclooxygenase mediated arachidonic acid metabolite) having several anti-inflammatory effects, including down-regulation of the cytokine response of both macrophages and lymphocytes (9). The native drug is a racemate of four stereoisomers, available as a viscous liquid form, which is difficult to formulate due to its chemical instability. Misoprostol rapidly de-esterifies to its active form, misoprostolic acid, after oral administration, and is excreted in urine as its inactive metabolites with an elimination half-life of approximately 30 min.

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MATERIALS AND METHODS

Synthesis of Polymer

Modified thermosensitive triblock co-polymer, (polyethylene glycol-poly[lactic acid-co-glycolic acid]-polyethylene glycol), PEG-PLGA-PEG, was synthesized by ring-opening polymerization according to the modified procedure previously described (4,5). Briefly, ring-opening polymerization of D,L-lactide (DLLA) and glycolide (GA) onto monomethoxy poly(ethylene oxide) mPEG 750 was done using 1% stannous octoate as a catalyst in toluene under reflux conditions at 150~170°C, followed by coupling of the resulting diblock copolymer (mPEG 750-PLGA) using HMDI as a coupling agent) in toluene at 60°C for 12 h, followed by reflux at 140°C for 6 h. The resulting PEG-PLGA-PEG triblock copolymers were dried under reduced pressure. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded at 30°C with a Bruker DPX-300 NMR spectrometer (Billerica, MA) operating at 300 MHz by using $CDCl_3$ as solvent. The molecular weight and molecular weight distribution of the triblock copolymers were monitored by GPC system with Waters 1515 isocratic HPLC pump and Waters 2410 refractive index detector (Milford, MA). The GPC data were calibrated with polystyrene standards with molecular weights in the range of 640~1,010,000. Styragel HR 4 and HR 2 columns (Waters) were used in series. The molecular weights were calculated using monodispersed polystyrene standard (Waters). All of the determinations were carried out in THF at a flow rate of 1.0 ml/min at 35°C. The molecular weight of diblock copolymers was controlled at around 3000–4500. PEG-PLGA-PEG triblock copolymers were synthesized, and its structure was confirmed by ^{1}H NMR. The weight average molecular weight (M_w) of triblock copolymer was 13,981 with a polydispersity index of 1.52 as determined by GPC. The aqueous solutions (30%, w/v) of this polymer flows freely at room temperature but forms a gel at 37°C (5).

Preparation of Solutions To Be Instilled

A 0.02% w/v solution of free FITC in 0.1 M phosphate buffer without any polymer was instilled in control animals of this study. The same amount of FITC in 0.1 M phosphate buffer pH 7.2 was added to a 30% w/v solution of PEG-PLGA-PEG polymer at room temperature by constant shaking in dark for 3 h.

Instillation into the Animals

Female Sprague-Dawley rats in the weight range of 200–250 g were used in the current study. Six animals were included in each group. Before intravesical instillation, animals were lightly anesthetized with halothane. Volume of instillation into rat bladder was kept at 0.5 ml of free FITC solution or FITC entrapped in hydrogel. Intravesical administration was done using a PE-50 tubing (Clay-Adams, Parsipanny, NJ, USA) inserted into the bladder through the urethra and tied in place by a ligature around the urethral orifice. Under continuing halothane anesthesia, the urethra was left tied for 30 min after instillation to prevent evacuation by the animal, as well as to allow enough time for sol-gel transformation to occur inside the bladder. All animals were given subcutaneous injection of antibiotic (Pen-strep, 30 mg/kg) to prevent any infection as a result of the procedure. All protocols involving the use of animals in this study adhered to "Principles of Laboratory Animal Care" (NIH Publication No. 85-23) and were approved by the IACUC of the University of Pittsburgh.

Urine Collection

After recovery from halothane anesthesia, rats were kept in metabolic cages with food and water *ad libitum* for next 24 h to study the kinetics of FITC excretion in the urine. Urine was collected at time points chosen arbitrarily. Cumulative urine output was measured from the volume of urine collected at these time points. Animals were sacrificed by carbon dioxide euthanasia, and bladders were removed.

Fluorescence Measurement

Collected urine was diluted several-fold to reduce the background contributed by other urine constituents. Fluorescence emitted by FITC was measured using Perkin Elmer spectrofluorimeter (Fremont, CA) at l_{Ex} = 496 nm and l_{Em} = 512 nm.

Efficacy Studies

Adult female Sprague-Dawley rats were injected intraperitoneally with a high dose of cyclophosphamide (100 mg/kg) under halothane anesthesia to induce cystitis (10). Chemical cystitis induced by cyclophosphamide is characterized by marked increase in erosions, ulcers in bladder including inflammatory cell infiltration, hemorrhages, and increased micturition frequency (11). Rats injected with cyclophosphamide were divided into four groups, and three of which served as controls were either instilled with saline, hydrogel, or misoprostol independently (n = 3). The main treatment group was instilled with hydrogel containing misoprostol. Due to the unstable nature of misoprostol in its native form, we used the drug available in commercially available Cytotec tablets from Searle (Chicago, IL). Tablets were homogenized to form a powder in mortar and pestle, and powdered tablet containing equivalent of 50 μg misoprostol was administered with or without hydrogel in 0.5-ml volume of instillation. Rats were kept in the metabolic cage after instillation for 24 h followed by cystometric measurement (CMG) under urethane anesthesia (1.2 g/kg, subcutaneous) to measure frequency of micturition.

CMG was done as previously reported from our lab (12). Briefly, PE50 tubing (Clay-Adams) was inserted into the bladder through the urethra, and, using a three-way stopcock, the catheter was connected to a pressure transducer for recording intravesical pressure and to a syringe pump for infusing saline into the bladder. The catheter system was filled with 0.9% w/v saline. After the bladder was emptied, a cystometrogram (CMG) was performed to measure micturition frequency, which was the number of voiding contractions occurring during a 60-min time period of transurethral saline infusion at the rate of 0.04 ml/min. Number of contractions (peaks) divided by time period (60 min) gave the number used to compare efficacy of treatment. After completion of CMG, bladders were isolated, fixed in Zamboni fixative, and then cryosectioned for staining with haemtoxylin and eosin (H&E).

Statistical Analysis

All values in the text and figures are mean values ± SEM. Student's *t* test was used to test for significance between unpaired groups.

RESULTS

Urine collected at various time points from rats in metabolic cages was used to calculate cumulative urine output over a 24 h time period (Fig. 1). Mean cumulative urine output of rats instilled with free FITC was 21.35 ± 2.73 ml and that of rats instilled with FITC entrapped in hydrogel was 19.55 ± 2.72 ml (number of animals in each group was 6). No statistically significant difference was detected between the groups. The fluorescence intensity of urine measured at different time points from the collected urine is plotted (Fig. 2a). Fluorescence intensity was normalized for the volume of urine collected at each time point. A significantly higher fluorescence signal was measured in the urine collected at the first time point of 2 h from the rats instilled with free FITC solution ($p < 0.01$). However, at the later time point of 8, 16, and 24 h, a higher signal was observed in the urine of rats instilled with FITC entrapped in hydrogel with statistical significance for later two time points ($p < 0.01$). Bladders from both groups were isolated 24 h after instillation for examination under ultraviolet light with the help of a dissecting microscope to detect the presence of any adhering FITC with the bladder. Bladders instilled with FITC entrapped in hydrogel exhibited bright green fluorescence of FITC whereas only background fluorescence was visible in the rats instilled with free FITC (Fig. 2b). The figure also serves as visual evidence for the formation of hydrogel inside the bladder after instillation, because the hydrogel was stained with the dye.

Therapeutic Effect with Intravesical Delivery of Misoprostol

Cystitis induced by injection of cyclophosphamide leads to urinary incontinence in rats marked by wet and dirty perineal region around urethra. Rats instilled with saline had similar appearance during their stay in the metabolic cage (data not shown). Rats kept in metabolic cages after being instilled with misoprostol entrapped in hydrogel appeared to be continent after cyclophosphamide injection. Cystometric measurement performed under urethane anesthesia evaluated the micturition reflexes induced by saline infusion with a transurethral catheter at a rate of 0.04 ml/min, which approximates the rate of physiological bladder filling with urine. Continuous filling of urinary bladder with saline elicits multiple contractions of bladder evident as number of micturitions (indexed by peaks in the cystograph). The number of peaks evoked over a 60-min infusion period describes micturition frequency, which was used to compare treatment groups with control groups. CMGs performed on rats 24 h after cyclophosphamide injection showed a dramatic decrease in the frequency of micturition in the rats instilled with misprostol in hydrogel (0.04167 ± 0.01014) compared to rats instilled with saline alone (0.1583 ± 0.04640, n = 3, $p < 0.05$) (Fig.3). Misoprostol alone (second tracing from top) and hydrogel without misoprostol (third tracing from top) offered only marginal improvement over saline instillation. Histological assessment done by H&E staining revealed severe ulcerative cystitis in the saline-instilled rats after cyclophosphamide injection (Fig. 4a). Epithelium was denuded leaving an ulcerated area with

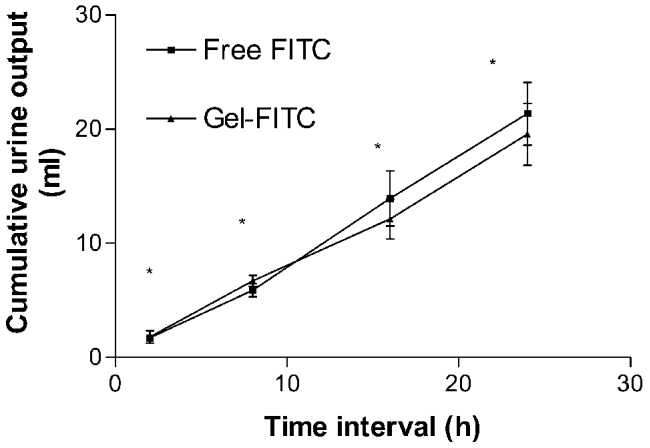

Fig. 1. Cumulative urine output of the rats instilled with free FITC solution (■) and FITC entrapped in a hydrogel (▲) formed by a thermosensitive polymer. Difference in the cumulative amount of urine excreted for 24 h after instillation was not significant (*$p > 0.05$) (n = 6).

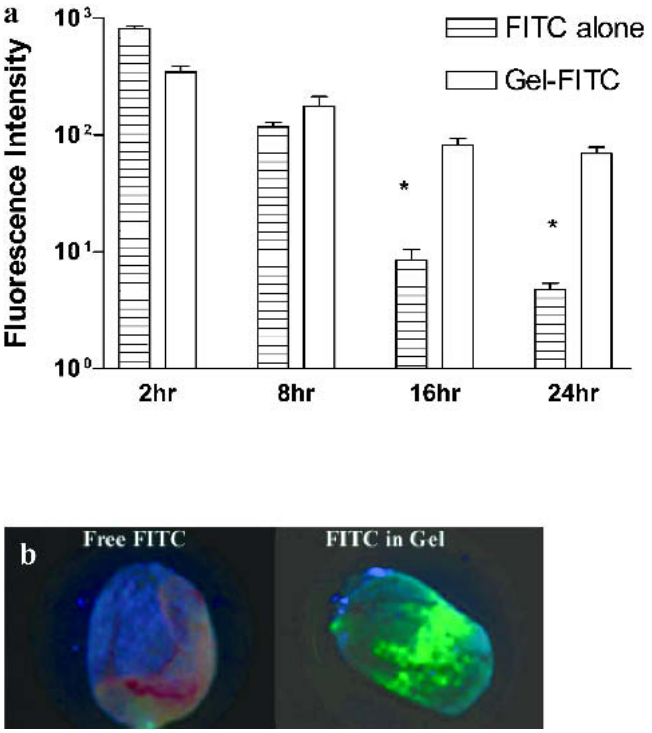

Fig. 2. (a) Semi-logarithmic plot of fluorescence intensity of the urine measured at various time points after instillation of free FITC and FITC entrapped in hydrogel. Urine was diluted several-fold before measuring the fluorescence of FITC at 512 nm in the Perkin Elmer spectrofluorimeter with excitation at 498 nm (mean ± SEM; n = 6), *$p < 0.01$. (b) Photographs of the representative rat bladders (after cut opened longitudinally) taken with dissecting microscope under ultraviolet light at 24 h after instillation of free FITC solution (left) and FITC entrapped in hydrogel (right). In contrast to the bright green fluorescence of FITC seen in bladder with hydrogel, only a background signal was visible with free FITC (left) (1× magnification).

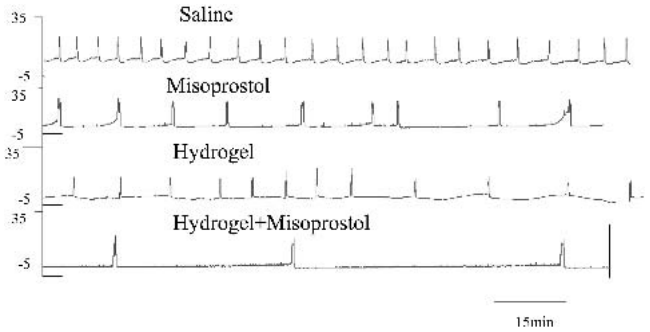

Fig. 3. Representative cystometry recordings illustrating the effect of intravesically delivered misoprostol in rats treated with cyclophosphamide. When the bladder was continuously filled, multiple contractions were elicited, and the number of micturitions (indexed by peaks in the cystograph) evoked over a 60-min infusion period was used to describe micturition frequency. Cyclophoshamide administration markedly increased micturition frequency as seen in the topmost panel representing saline-treated rats. Cyclophosphamide-induced increased micturition frequency was dramatically reduced in the rats instilled with misoprostol entrapped in hydrogel (lowermost panel). Second and third panel from the top are from rats treated with misoprostol in saline and hydrogel alone, respectively. Scale on vertical axis is intravesical pressure in cm of water (cm/w) and time is on the horizontal axis.

submucosal edema, inflammation, and vascular ectasia and congestion. In tissue sections obtained from rats treated with misoprostol (Fig. 4b) and hydrogel separately (Fig. 4c), hisotological changes were less severe, which included infiltration of inflammatory cells and edema of subepithelial tissue layer responsible for its slightly enlarged appearance. Treatment with misoprostol in hydrogel showed drastic improvement, which was evident by lack of any ulcerations in the normal appearing epithelium with slightly enlarged appearance (Fig. 4d).

DISCUSSION

Intravesical drug delivery continues to remain an ideal treatment option for therapy of superficial bladder cancer, because drug exposure is restricted only to the disease site and systemic exposure to toxic anticancer drugs is avoided (1). In recent years, the intravesical route has been exploited for effective therapy of other bladder diseases such as neurogenic bladder (13). Instillation of C-fiber neurotoxin into the neurogenic bladder of spinal cord injury patients limits the potent action of capsaicin only to the afferent fibers in the bladder wall and circumvents its possible systemic neurotoxicity (14).

However, conventional vehicles used for the intravesical route of administration fail to provide a sustained exposure of drug to the urothelium. Duration of exposure for instilled drugs does not last beyond the first voiding of urine after instillation. Increase in efficacy of drugs used for intravesical therapy would be expected if the duration for direct contact between the drug and the abnormal urothelium were increased (15). Polymers that display a physicochemical response to stimuli have been widely explored as potential drug delivery systems. Therefore, in our current work, we explored the effectiveness of a modified PEG-PLGA-PEG polymer as a feasible hydrogel matrix for sustained delivery by the intravesical route. The thermosensitive polymer used in our study offers several advantages over common drug delivery systems. First, the formulation is simple and requires no organic solvent. Second, the products from bioerosion of this nontoxic biocompatible polymer are polyethylene glycol (PEG), glycolic acid, and lactic acid, all of which are nontoxic, making the polymer biocompatible (16). It was found that water penetration within the hydrogel changes with the change in hydrophilic PEG content of the polymer (17).

We observed no significant difference in urine output with the treatment of hydrogel during the 24-h period (Fig. 1), indicating that the soft gel did not obstruct the elimination of urine from the bladder. However, the difference observed in the cumulative urine output between two groups did not prove to be statistically significant. The picture shown in Fig. 2b provides the most plausible explanation for the data shown in Fig. 1. The photograph of the bladder taken 24 h after instillation indicates that hydrogel did not completely fill the bladder, rather it attached itself as a smooth layer on the inner surface of the bladder. The presence of the gel inside the bladder after instillation of a free-flowing liquid indicates that this vehicle could produce a prolonged drug exposure to the urothelium, even after multiple voidings postinstillation of the hydrogel.

It follows naturally from the retention inside the bladder of hydrogel delivery system that it can withstand the hostile environment of rat urinary bladder. The hydrogel could also resist being washed away by the flush of urine during the multiple voidings that occurred during 24 h. The higher fluorescence signal in the control group at the initial time point of 2 h demonstrates the drawback of conventional vehicles used for intravesical drug delivery (Fig. 2a). Animals instilled with FITC entrapped inside a hydrogel showed a lower fluorescence at the early time point presumably because FITC did not exist in the bladder as free FITC, but rather it was sequestered in the hydrogel and was released over a period of time.

According to earlier reports on release profile of drugs contained in the sol-gel transition system, it is probable that FITC is released at first by diffusion, and later by the combination of both diffusion and degradation mechanisms (18). The reversible nature of gelation process of a thermosensitive hydrogel may also facilitate easy removal of the gel from the bladder if desired at any time after administration by simply rinsing the bladder with sterile water at room temperature. A lower temperature of instilled water should convert from the gel state of the polymer back to its sol state allowing it to be voided in the urine.

To answer the question, whether a drug entrapped in the hydrogel is available for its therapeutic action, we tested intravesical misoprostol in a rat model of cyclophosphamide cystitis. A previous study done on the same model reported reduction in cyclophosphamide induced ulceration, inflammation, and edema in bladder walls of male rat following long term oral administration of misoprostol (19). A daily oral dose of 600 μg administered chronically for 3 months was also effective in patients with refractory interstitial cystitis (20). In this study, we chose to use a fraction of the oral dose of misoprostol for a single intravesical administration in the same rat model of cystitis. Urinary incontinence induced by cyclophosphamide was observed during the 24-h metabolic cage stay of the rats. Simultaneous instillation of misoprostol

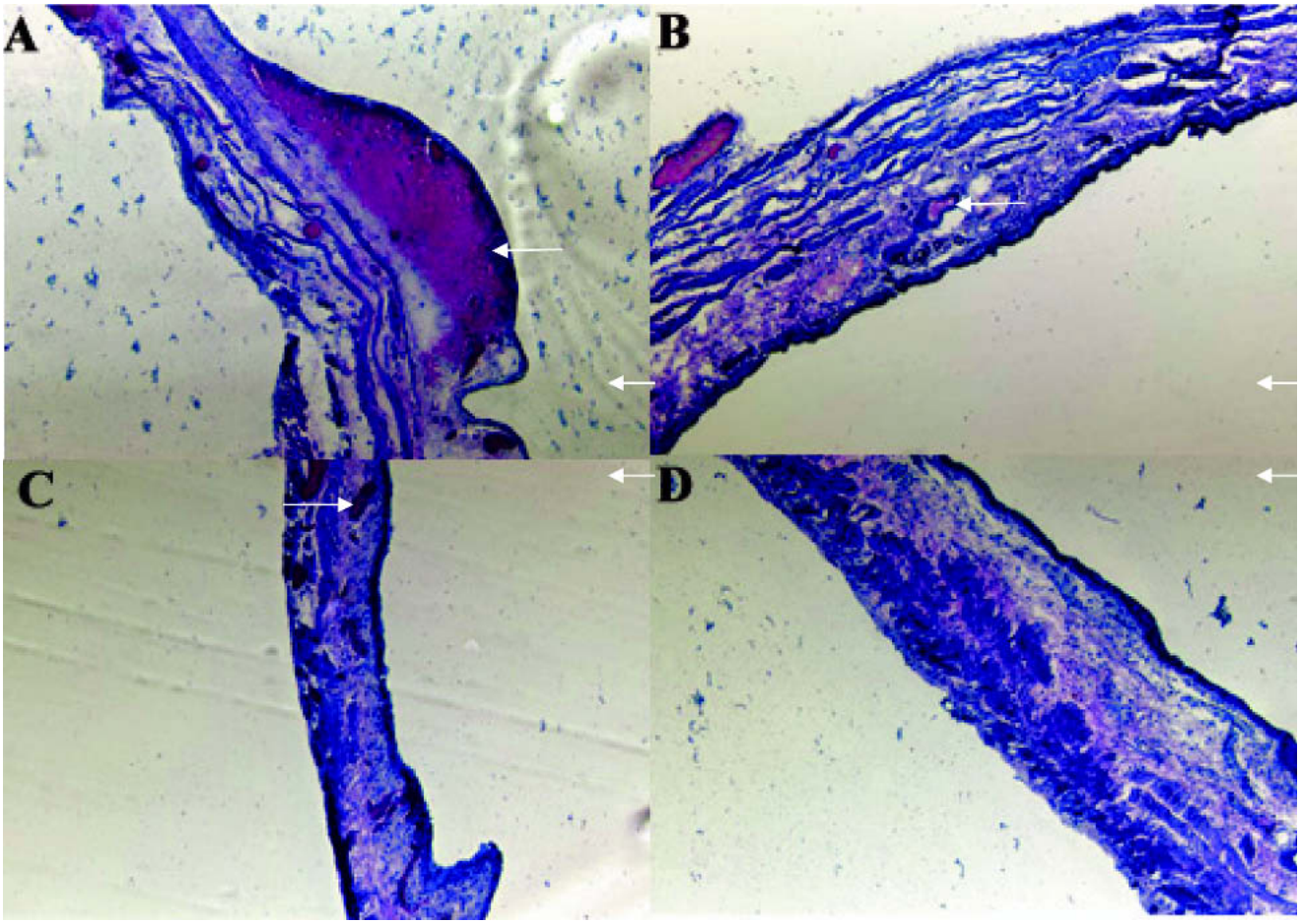

Fig. 4. Hemtoxylin & eosin–staining cyclophosphamide-treated rat bladders in representative cross sections: Top-left panel A shows severe lesions in the epithelium, extensive submucosal edema, and multiple bleeding vessels in subepithelium in saline instilled rats. Sections in top-right panel B and lower left panel C were from rats instilled with misoprostol and hydrogel *alone*, respectively, showing changes such as edema, moderate vascular ectasia, and vascular congestion compared to saline-treated rats. Lower right panel D reveals the resistance afforded by misoprostol entrapped in hydrogel from the chemical cystitis induced by cyclophosphamide with only slightly enlarged subepithelial layer due to edema (lumen side and bleeding spots are marked by white arrow in all sections and magnification is 10×).

in hydrogel enabled rats to maintain continence following intraperitonal injection of cyclophosphamide. Cystometric measurements showed that misoprostol entrapped in hydrogel is available for its biological action as is evident from the decreased bladder contraction frequency in misoprostol treated rats (Fig. 3). Rats instilled with hydrogel without misoprostol offered improvement in micturition frequency over saline instillation, and benefit was comparable to rats instilled with suspension of misprostol (tablet powder). The beneficial effect observed in rats instilled with hydrogel itself without any drug might be a consequent of its emollient properties as hydrogel with similar composition have shown excellent wound dressing activity (21). It is possible that intravesically administered hydrogel might help in healing of ulcers induced by cyclophosphamide, and wound-healing effect of hydrogel might underlie the benefit seen in Fig. 3. As previously reported from our lab, in absence of cyclophosphamide injection, instillation of hydrogel in normal rats did not significantly change micturition frequency compared to rats instilled with saline (22). Effect of misoprostol alone in improving micturition frequency is expected from the local action of PGE1 analog on the urothelium mucosa. Histological examination (Fig. 4) seems to support our explanation for Fig. 3 as well as affirms that the uroprotective action of misoprostol is retained when delivered intravesically using a hydrogel in rat model of cystitis. Results of our efficacy study agree with observation reported from another lab following oral administration of misoprostol in cyclophosphamide cystitis (19).

CONCLUSIONS

This paper describes the first successful use of a modified thermosensitive PEG-PLGA-PEG polymer as a suitable matrix for sustained drug delivery by intravesical route. Modifications introduced into the polymer in our lab enable the sol-gel transformation of this thermosensitive polymer to occur even in the presence of typical urine constituents, urea, and excess electrolytes to extend its application for intravesical therapy of bladder diseases. Safe and long-acting intravesical delivery of misoprostol may be a desirable new treatment of interstitial cystitis. However, further safety studies of the polymer following intravesical instillation are needed before it becomes a clinically feasible drug delivery system.

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