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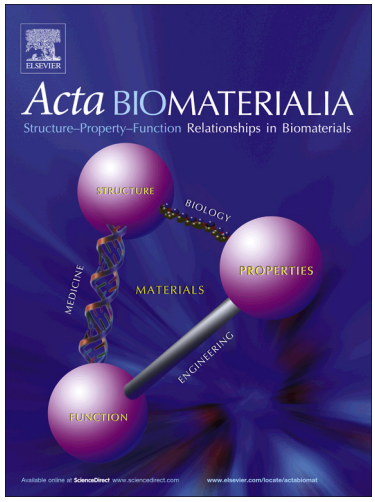

Full length article

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PII: S1742-7061(18)30593-2
DOI: https://doi.org/10.1016/j.actbio.2018.10.003
Reference: ACTBIO 5708

To appear in: *Acta Biomaterialia*

Received Date: 2 March 2018
Revised Date: 16 August 2018
Accepted Date: 3 October 2018

Please cite this article as: Kim, S., Leandre Traore, Y., Ho, E.A., Shafiq, M., Kim, S-H., Liu, S., Design and development of pH-responsive polyurethane membranes for intravaginal release of nanomedicines, *Acta Biomaterialia* (2018), doi: https://doi.org/10.1016/j.actbio.2018.10.003

Design and development of pH-responsive polyurethane membranes for intravaginal release of nanomedicines

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ABSTRACT

The objective of this study was to develop and characterize a novel intravaginal membrane platform for pH-triggered release of nanoparticles (NPs), which is essential for efficient

intravaginal delivery of certain effective but acid-labile therapeutic agents for sexually transmitted infections, such as small interfering RNA (siRNA). pH-responsive polyurethane (PU) was electrospun into a porous nanofibrous membrane. The diameters of the fibers, as well as the thickness and pore sizes of the membrane under dry and wet conditions (pH 4.5 and 7.0), were determined from scanning electron microscopy (SEM) micrographs. pH-dependent zeta-potential (ζ) of the membrane was evaluated using a SurPASS electrokinetic analyzer. Visiblex™ color-dyed polystyrene NPs (PSNs, 200 nm, -COOH) and CCR5 siRNA-encapsulated solid lipid NPs (SLNs) were used for *in vitro* NP release studies in a vaginal fluid simulant (VFS) at pH 4.5 (normal physiological vaginal pH) and 7.0 (vaginal pH neutralization by semen). During 24 hours of incubation in VFS, close-to-zero PSNs (2 ± 1%) and 28 ± 4% SLNs were released through the PU membrane at pH 4.5, whereas the release of PSNs and SLNs significantly increased to 60 ± 6% and 59 ± 8% at pH 7.0, respectively. The pH-responsive release of NPs hinged on the electrostatic interaction between the pH-responsive membranes and the anionic NPs, and the change in pH-responsive morphology of the membrane. *In vitro* biocompatibility studies of the membranes showed no significant cytotoxicity to VK2/E6E7 human epithelial cells and Sup-T1 human T-cells and no significant changes in the expression of pro-inflammatory cytokines (IL-6, IL-8, and IL-1β). Overall, these porous pH-responsive PU membranes demonstrated their potential in serving as "window" membranes of reservoir-IVRs for pH-responsive intravaginal release of NPs.

***Keywords*:** pH-responsive release of nanoparticles; smart intravaginal delivery; pH-responsive polyurethane; electrospun pH-responsive membrane; controlled drug delivery

1. Introduction

More than 30 million people globally live with human immunodeficiency virus (HIV) infection that can potentially progress to acquired immunodeficiency syndrome (AIDS) [1,2]. HIV is a life-threatening disease, and no cure has been found to date. Prevention and early treatment are important for decreasing the prevalence and incidence of HIV.

Intravaginal drug delivery is beneficial for HIV prevention because unprotected heterosexual intercourse is the typical route of HIV transmission. Moreover, the advantages of intravaginal drug delivery include increased drug delivery efficiency to the target site, drug bioavailability, pharmacological response, and drug compliance of patients [3,4]. Intravaginal formulations such as ointments, creams, gels, films, vaginal tablets, and intravaginal rings (IVRs) have been developed for the release of drugs [5]. Among intravaginal formulations, IVRs have advantages over other vaginal formulations because they can provide sustained controlled drug release for a prolonged period [6]. The availability of the intravaginal formulations could greatly empower women to protect themselves from infection because the application of formulations does not require the consent of their partner [7].

Anti-HIV drugs and small interfering ribonucleic acids (siRNAs) act on T cells at different stages of the HIV life cycle, including receptor interaction, virus–cell fusion, reverse transcription, integration, and proteolytic processing to block the transmission progress [8,9]. Hydrophilic anti-HIV drugs have advantages because of their solubility in an aqueous environment in comparison to the poorly water-soluble anti-HIV drugs. However, it is cumbersome to achieve a high delivery efficiency of hydrophilic anti-HIV drugs to T cells because the drugs cannot effectively penetrate through the vaginal mucus barrier and cross the lipid bilayer of the cell membrane [10,11]. On the other hand, poorly water-soluble anti-HIV drugs have a limitation in their application because of their relatively low solubility and

bioavailability in an aqueous environment [12]. Similarly, siRNAs have a high potential to prevent HIV transmission by simultaneously targeting host genes and viral genes [13], but they have low stability in the acidic vaginal environment.

In this regard, an alternative method is needed to deliver anti-HIV drugs and siRNAs to increase the delivery efficiency and reduce side effects [14]. Drug delivery systems (DDS) using nanocarriers such as micelles, vesicles, solid lipid nanoparticles (SLNs), and liposomes have been developed for the delivery of drugs and genes [15,16]. A nanocarrier can improve dispersion of poorly water-soluble anti-HIV drugs, protect cargos from the acidic conditions, and increase delivery efficiency [17,18]. DDS using nanocarriers can also reduce systemic toxicity and off-target effects of HIV treatment [19].

Although nanocarriers are beneficial for the intravaginal delivery of anti-HIV drugs and siRNA, a continuous release of drugs and siRNAs is not desirable. For prevention of HIV, it is desirable for the anti-HIV drug- or siRNA-loaded nanocarrier to be released only during the heterosexual intercourse to avoid unnecessary exposure to drug and reduce side effects. Therefore, it is necessary to release nanocarriers from IVRs upon pH change from 4.5 to 7.0 because vaginal pH can be increased to neutral pH from normal acidic vaginal pH (pH 3.5-4.5) by the introduction of the seminal fluid during heterosexual intercourse [20–24].

Our research group fabricated solvent-cast pH-responsive PU membranes to serve as "window" membranes of reservoir-IVR [25]. The solvent-cast membranes showed a continuous release of the hydrophilic model drug "diclofenac sodium" (NaDF) at pH 7.0 but close-to-zero release of NaDF at pH 4.5. Although the solvent-cast pH-responsive PU membranes demonstrated on-demand drug release, the membranes failed to achieve pH-responsive permeation of nanoparticles (NPs) due to the insufficient interconnected space for NPs. In this

study, our research group proposed the fabrication of electrospun pH-responsive interconnected porous PU membranes as "window" membranes of reservoir-IVR for on-demand release of NPs.

Stimuli-responsive membranes have been developed for various types of applications including drug delivery devices and separation processes [26]. For example, a thermo- and pH-responsive composite membrane composed of poly(N-isopropylacrylamide-co-methacrylic acid) was investigated for permeability change triggered by environmental stimuli [27]. The permeability of vitamin B_{12} was increased with increasing temperature or decreasing pH. The pH-responsive permeation change of vitamin B_{12} was supported by change in pore size of the membrane due to swelling and shrinking of the polymer chains. Similarly, a pH-responsive electrospun membrane was fabricated using a mixture of poly(vinyl alcohol) and poly(acrylic acid) [28]. The membrane demonstrated a pH-responsive change in swelling ratio and thickness increase with increasing pH. Likewise, a pH-responsive gating membrane system with a pumping effect consisting of a porous membrane and cross-linked hydrogel has also been studied [29]. The porous membrane was fabricated using PVDF and conjugated with PMAA chains on its surface. Moreover, the hydrogel is prepared by plasma-graft pore-filling polymerization of poly(N,N-dimethylaminoethyl methacrylate) (PDM) for the inside of the reservoir as a pumping material. When pH is lower than the pKa of PMAA (4.65–5.35) and protonated PDM (4.5–5.5), the release is accelerated by the shrinking of PMAA chains and the swelling of the PDM hydrogel. In addition, a pH-responsive switchable membrane was fabricated by the self-assembly of polystyrene-b-poly(4-vinylpyridine) in the presence of metal ions [copper(II), cobalt(II), nickel(II), and iron(II)] and by nonsolvent-induced phase separation [30]. This membrane demonstrated change in pH-responsive pore size (larger pore size at pH 10.0 than at pH 2.0), water flux change, and permeation of polyethylene glycol (PEG; Mw 1,500-10,000) (higher

permeation at pH 7.0 than at pH 2.0). However, to the best of our knowledge, no study has reported pH-responsive release of NPs from a reservoir.

In the present study, a pH-responsive interconnected porous PU membrane was developed for smart intravaginal release of NPs. The pH-responsive PU membrane was fabricated by electrospinning to facilitate the fabrication of thin interconnected porous membranes. The permeability of a polystyrene model NP and a CCR5 siRNA-loaded SLN through the electrospun porous membrane was controlled by changing the pH-responsive morphology of the membrane and electrostatic interaction between the membrane and the NPs. Therefore, this is the first study to develop a pH-responsive membrane for stimuli-responsive intravaginal release of NPs to achieve smart drug delivery. The pH-responsive interconnected porous PU membrane has potential utility for drug delivery from medical devices including IVRs.

2. Materials and methods

2.1. Materials

1,1,1,3,3,3-Hexafluoro-2-propanol (HIFP, 99.99%), 1,4-Bis(2-hydroxyethyl)piperazine (HEP, 99%), 1,6-hexanediol (HD, 99%), 4,4′-Methylenebis(phenyl isocyanate) (MDI, 98%), anhydrous 1,2-dichloroethane (DCE, 99.8%), anhydrous diethyl ether (>99%), anhydrous N,N-dimethylformamide (DMF, 99.8%), dibutyltin dilaurate (DBTDL, 95%), dimethyl sulfoxide (DMSO, ≥99.5%), propylene glycol (PG, >99.5%), and tetrahydrofuran (THF) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Polyethylene glycol (Mw = 6,000) was purchased from EMD Chemicals (Mississauga, ON, Canada). Visiblex™ color-dyed polystyrene nanoparticles (PSNs, 200 nm, COOH on its surface, media: 0.1% Tween 20 in deionized water) was purchased from Phosphorex Inc. (Hopkinton, MA, USA). C_{12}-HPC(2-(4,4-Difluoro-5-

Methyl-4-Bora-3a,4a-Diaza-s-Indacene-3-Dodecanoyl)-1-Hexadecanoyl-sn-Glycero-3-Phosphocholine) was purchased from Thermo Fisher Scientific (Asheville, NC, USA). Vaginal fluid simulant (VFS) was prepared by dissolving 1.0 g acetic acid, 0.018 g bovine serum albumin, 0.222 g $Ca(OH)_2$, 0.6 g glycerol, 5.0 g glucose, 1.4 g KOH, 2.0 g lactic acid, 3.51 g NaCl, and 0.4 g urea in 1 L of distilled water. The pH of VFS was adjusted by adding 0.1 N HCl or 0.1 N NaOH solution [31].

2.2. Synthesis of pH-responsive polyurethane copolymers

pH-responsive and non-responsive polyurethane (PU) copolymers were synthesized and purified according to a procedure reported previously [25]. Briefly, a three-neck round-bottom flask was set up with nitrogen inlet–outlet purging to synthesize pH-responsive PU (PEG-HEP-MDI-PG). PEG and 1,4-Bis(2-hydroxyethyl)piperazine (HEP) (feed mole ratio of PEG: HEP = 0.1:0.9) were dissolved in anhydrous 1,2- dichloroethane and added to the reactor. A catalytic amount of DBTDL and a stoichiometric ratio of diisocyanate (MDI, feed mole ratio of OH/NCO = 1) were added to the reactor. The reactor was maintained at 60 °C for 3 h with stirring under a nitrogen atmosphere. After the reaction, PG (feed mole ratio of HEP:PG = 0.9:1.0) and a stoichiometric ratio of diisocyanate (MDI, OH/NCO = 1) were added. The reactor was maintained at 60 °C for 3 h under nitrogen atmosphere. The product was then precipitated in 8-fold diethyl ether and washed with excess diethyl ether. The product was redissolved in DMSO, reprecipitated in 8-fold distilled water, and washed with excess distilled water several times to remove remaining impurities. HD was used to synthesize control non-pH-responsive PU (PEG-HD-MDI-HD) instead of HEP and PG. The overall feed mole ratio of raw chemicals for PEG-HDI-MDI-HD was PEG:HD:MDI = 0.1:2.9:3.0. The viscosity average molecular weight (Mv) of the synthesized PEG-HEP-MDI-PG and PEG-HD-MDI-HD copolymer was calculated from the

Mark–Houwink equation ($[\eta] = 3.64 \times 10^{-4} M^{0.71}$) for the polyether-PU in DMF at 25 °C [32]. A Cannon–Ubbelohde dilution viscometer was used to measure viscosities in a constant temperature bath set at 25.0 ± 0.5 °C. Pure DMF and 25 mg of PU in 10 mL of DMF were prepared for measurement. The PU solution was diluted from 2.50 mg/mL to 1.25 mg/mL. Travel times of pure DMF and polymer solutions between two measurement marks were recorded. Mv values of PEG-HEP-MDI-PG and PEG-HD-MDI-HD were 1.23×10^6 and 2.36×10^6, respectively.

2.3. Electrospinning of pH-responsive polyurethane copolymers

pH-responsive PU (PEG-HEP-MDI-PG) was electrospun to fabricate fibrous, interconnected, and porous PU membranes using an electrospinning apparatus (Ne300, Inovenso, Istanbul, Turkey). Different mixing ratios of DMF and THF were applied to determine the most suitable electrospinning conditions for fabricating a fibrous pH-responsive PU membrane. The viscosity of the pH-responsive PU solution was evaluated using an Advanced Rheometer (AR 2000, TA Instruments) and was calculated from the relationship between shear stress and shear rate at 0.1-100 rad/s using 20 mL of 20 wt% PU solutions. The concentration of PU in mixed solvents (20 wt%), voltage applied on the nozzle (30 kV), feeding speed of the pH-responsive PU solution (0.7 mL/h), nozzle-to-collector distance (15 cm), and dispensed volume of the PU solution (1 mL) were all fixed for electrospinning. These electrospun pH-responsive PU membranes were washed with excess distilled water to remove remaining impurities and then dried at room temperature.

Control PU (PEG-HD-MDI-HD) membranes were fabricated using a custom-designed electrospinning setup with the following conditions: 9% w/v solution in HFIP, needle-to-collector distance of 18 cm, flow rate of 1 mL/h, voltage of 15 kV, and a 21-gauge needle. The

needle was clamped to the positive electrode of a high-voltage power supply (ESP200D, NanoNC, Seoul, South Korea), whereas the negative electrode was connected to a rotating aluminum drum collector (250 rpm). The solution was delivered using a syringe pump (ESP200D). Electrospun nanofibers were dried in a vacuum oven at ambient temperature for 3 days and stored in a desiccator for subsequent use.

Scanning electron microscope (SEM; FEI Quanta ESEM) images of the fabricated PU membranes were captured to evaluate the diameter (DI) of fibers, pore size, and thickness of the membranes [33–36]. Membrane samples were coated with 10 nm Au-Pd before capturing images. The DI, pore size, and thickness were manually measured using ImageJ, a free photo editing software program, from SEM images using their size bar as reference [37]. The threshold of pores was set to the blackest area versus the gray area using SEM images at ×1000 magnification.

2.4. Physicochemical characterization of electrospun porous pH-responsive PU membrane

Changes in the pH-responsive morphology (pore size, thickness, and DI) of electrospun PU membranes were evaluated from SEM images. For evaluation, electrospun porous pH-responsive (DMF:THF = 3:7) and control PU membranes were cut into 2 × 2 cm squares and immersed in VFS at pH 7.0 or pH 4.5 at 37 °C for 3 h with gentle shaking. Swollen samples were extracted from VFS and kept at -80 °C followed by lyophilization. Fracture surfaces of these electrospun PU membranes for cross-section imaging were prepared by cracking membranes under liquid nitrogen.

The streaming current was measured for the electrospun PU membranes using a SurPASS electrokinetic analyzer (Anton Paar) at pH ranging from 3.5 to 8.5 [38–40]. Rectangular membrane samples (20 mm × 10 mm) were prepared and attached to sample holders using a

double-sided adhesive tape. The measurement was taken with an adjustable-gap cell, and the flow channel gap was set at 100 μm. As a background electrolyte, 1 mM KCl solution was used, and the pH was adjusted using 0.05 M HCl or 0.05 M NaOH solution. Zeta-potential (ζ) of the membranes was automatically calculated using the following Helmholtz–Smoluchowski equation.

$$\zeta = \frac{dI}{dP} * \frac{\mu}{\varepsilon\varepsilon_0} * \frac{L}{A}$$

where dI/dP is the slope of the streaming current versus pressure, μ is the solution dynamic viscosity, ε is the dielectric constant of the solution, ε_0 is the vacuum permittivity, L is the streaming channel length, and A is the cross-section of the streaming channel.

2.5. Nanocarrier permeability studies using a model nanoparticle (PSNs, -COOH)

Commercially available blue-dyed PSNs (-COOH) were used for conducting pH-responsive NP release tests. Before release studies, the average particle size of PSNs was evaluated by dynamic light scattering (DLS) using a ZetaPALS potential analyzer (Brookhaven Instrument, Holtsville, NY, USA). Then, PSNs 0.1 μg/mL in VFS at pH 4.5 and 7.0 were prepared by vortex shaking for 3 min at 3,000 rpm. The pH of PSN suspension in VFS was adjusted by adding 0.1 N HCl or NaOH solution. A scattering angle of 90° and laser light at 659 nm were applied for DLS measurement.

The ZetaPALS potential analyzer was used to evaluate the zeta-potential of PSNs. Two suspensions of PSNs (0.1 μg/mL) in distilled water (one at pH 4.5 and another at pH 7.0) were prepared. The pH of the suspension was adjusted using 0.1 N HCl or 0.1 N NaOH solution. A Smoluchowski model was used to evaluate surface charge.

pH-responsive NP release studies were performed using Franz cells. Briefly, electrospun porous pH-responsive (DMF: THF = 3: 7) and control PU membranes were cut into 2×2 cm

squares and sandwiched between the upper and bottom chambers of Franz cells. Three suspensions of PSNs at 1 mg/mL (at pH 4.5, pH 5.5, and pH 7.0) in VFS were prepared for the upper chamber of Franz Cells. The bottom chamber was filled with VFS of pH same as that in the upper chamber. The bottom chamber was kept at 37 °C. The VFS in the bottom chamber was gently stirred with a magnetic bar. After adding 800 µL of the prepared PSN suspension to the upper chamber of Franz cells, 300 µL of the sample solution was collected to evaluate released amounts of PSNs at 1, 2, 3, 6, 12, and 24 h from each arm of Franz cells. The amount of PSN released was quantified by reading absorbance at 300 nm using a plate reader.

The amounts of PSNs associated with electrospun porous PU membranes (DMF:THF = 3:7) in VFS at pH 4.5, 5.5, and 7.0 were evaluated. Round-cut pH-responsive and control PU membranes (DI: 0.8 cm) corresponding to an area of 0.5024 cm^2 between the upper and bottom chambers of Franz cells were prepared. Prepared electrospun PU membrane samples were immersed in 6.4 mL of 0.125 mg/mL PSN suspension at 37 °C with gentle horizontal shaking (100 rpm) because 0.8 mL of 1 mg/mL PSNs was used for the upper chamber, whereas 5.6 mL of the release medium (in VFS at pH 4.5, 5.5, or 7.0) was used for the bottom chamber of Franz cells to evaluate the pH-responsive NP release. At each time point (1, 2, 3, 6, 12, and 24 h), 300 µL of the sample solution was taken to evaluate the amount of PSNs associated with electrospun PU membranes (DI: 0.8 cm). Fresh VFS was replenished for the suspension. The concentration of free PSNs was quantified by measuring absorbance at 300 nm using a plate reader. The percentage of PSNs associated with the membranes was back-calculated using the concentration of free PSNs.

2.6. Nanocarrier permeability studies using CCR5 siRNA-encapsulated solid lipid nanoparticles (SLNs)

CCR5 siRNA-encapsulated SLNs were prepared by the emulsion solvent diffusion method described elsewhere, with slight modification [41]. Briefly, 2.5 mg of glyceryl monostearate (Sigma-Aldrich, MO, USA), 5 mg of ∝-L-phosphatidylcholine (Soy-95%) (Avanti Polar Lipids, AL, USA), and 20 µg of fluorescently labeled BODIPY-phosphocholine(C_{12}-HPC(2-(4,4-Difluoro-5-Methyl-4-Bora-3a,4a-Diaza-s-Indacene-3-Dodecanoyl)-1-Hexadecanoyl-sn-Glycero-3-Phosphocholine) were dissolved in 700 µL of acetone and ethanol heated at 55 ºC. Twenty-five micrograms of CCR5 silencer siRNA (Thermo Fisher Scientific, Asheville, NC, USA) was condensed using polyethylene imine and added to the dissolved lipid mixture. The mixture was sonicated for 20 s, thereby resulting in the formation of the first emulsion. The first emulsion was emulsified with 2% polyvinyl alcohol (8 mL) (Sigma-Aldrich, MO, USA) and sonicated for 60 s. The NPs were stirred for 8 h to evaporate the organic solvent and were collected by centrifugation. The supernatant was collected to determine the encapsulation efficiency, and the SLNs were washed 3 times. The SLNs were resuspended in mannitol (1:1 ratio w/w) and freeze-dried.

pH-responsive release studies were performed using the CCR5 siRNA-encapsulated SLNs. Briefly, electrospun porous pH-responsive (DMF:THF = 3:7) membranes were cut into 2 × 2 cm squares and sandwiched between the upper and bottom chambers of Franz cells. Before use, the freeze-dried CCR5 siRNA-encapsulated SLNs were resuspended in distilled water at 1 mg/mL and washed with distilled water three times to remove mannitol. Two suspensions of CCR5 siRNA-encapsulated SLNs at 1 mg/mL (at pH 4.5 or pH 7.0) in VFS were prepared for the upper chamber of Franz Cells. The bottom chamber was filled with VFS of the same pH as that in the upper chamber and was kept at 37 °C. After adding 800 µL of the prepared CCR5 siRNA-encapsulated SLN suspension to the upper chamber of Franz cells, 300 µL of the sample

solution was collected to evaluate released amounts of CCR5 siRNA-encapsulated SLNs at 1, 2, 3, 6, 12, and 24 h from each arm of Franz cells. The amount of CCR5 siRNA-encapsulated SLNs released was quantified by reading fluorescence at excitation 544 nm and emission 590 nm using a plate reader.

2.7. In vitro biocompatibility studies of electrospun porous pH-responsive PU membrane

The electrospun porous pH-responsive PU membrane (DMF:THF = 3:7) was evaluated on the vaginal epithelial cells VK2/E6E7 and the immune cells Sup-T1 for its cell toxicity and also for the production of the pro-inflammatory cytokines. To assess cytotoxicity, we used the elution assay method followed by the cell proliferation assay MTS (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega Corporation, Madison, WI, USA) because the elution test methods are widely applied for *in vitro* cytotoxicity testing of biocompatible materials including PU [42–44]. Briefly, 50 mg of the electrospun membrane sterilized in 70% isopropanol for 10 s, dried, and exposed to UV light for 1 h was incubated in 2.5 mL of either VK2/E6E7 cell medium consisting of keratinocyte serum-free medium (K-SFM) containing 0.1 µg/mL recombinant human epidermal growth factor, 50 mg/mL bovine pituitary extract, 0.4 mM of calcium, and 1% of penicillin/streptomycin or Sup-T1 cell medium consisting of RPMI-1640 containing 10% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin. The polymer was incubated for 1, 7, 15, and 30 days at 37 °C in an orbital shaker set at 100 rpm. At different time points, all the media were collected aseptically and used to treat 2.5 x 10^5 VK2/EE67 cells or Sup-T1 cells seeded in a 96-well plate and maintained at 37 °C under 5%

CO_2 for 24 h. Blank K-SFM or RPMI-1640 medium was used as negative the control and 1 M acrylamide prepared in K-SFM or RPMI-1640 was used as the positive control.

In addition, 50 μg/mL of lipopolysaccharide (LPS; Thermo Fisher Scientific, MA, USA) and 200 μg/mL of nonoxyl-9 (N-9, Spectrum Chemical, Corp., New Brunswick, NJ, USA) were prepared with K-SFM or RPMI-1640 and used to treat VK2/E6E7 cells or Sup-T1 cells as the positive control for the pro-inflammatory markers interleukin-6 (IL-6), interleukin-8 (IL-8) (induce by LPS), and interleukin-1 beta (IL-1β) (induced by N-9). After 24 h of treatment, the supernatant in each well was collected for a downstream ELISA assay for evaluating the pro-inflammatory markers and was replenished with fresh medium. Twenty microliters of the MTS reagent was added in each well, and the plate was incubated as specified in the MTS assay protocol, and cell viability was analyzed by reading the absorbance at 450 nm using a Synergy HT Multiplate Reader (BioTek, Winooski, VT, USA). ELISA kits obtained from R&D Systems (Minneapolis, MN, USA) were used to evaluate IL-6, IL-8, and IL-1β production. The absorbance of each ELISA plate was read at 450 nm using a microplate reader.

2.8. Statistical analysis

Data are presented as mean ± standard deviation (SD). The number of replicates is indicated as the *n*-value. One-way analysis of variance was performed on all results, with $P < 0.05$ considered as significant.

3. Result and discussion

3.1. Preparation of electrospun porous pH-responsive PU membrane

Interconnected porous PU membranes were fabricated by electrospinning. Various mixing ratios of DMF and THF (10:0, 7:3, 5:5, and 3:7) were applied to optimize the electrospinning condition of pH-responsive PU membranes (Fig. 1A-D). Electrospinning parameters such as the concentration of PUs, the voltage applied on the nozzle, feeding speed of the PU solution, nozzle-to-collector distance, and dispensed volume of the pH-responsive PU solution were fixed. The electrospun fibrous structure was fabricated with fine DIs when the DMF ratio was decreased in the mixed solvent (Fig. 1). Membranes fabricated by using a higher concentration of DMF (DMF:THF = 10:0 and 7:3) exhibited beaded fibrous structures (Fig. 1A-B); however, a uniform fibrous structure was observed for the membranes with an increase in the THF content (DMF:THF = 5:5 and 3:7) (Fig. 1C-D). The quality and DI of electrospun fibers depend on the boiling point of the solvent. As THF has a lower boiling point, it can evaporate more rapidly than DMF, thus resulting in an increased amount of fine fibers with an increase in the THF ratio in the mixed solvent [45,46].

Pore size and DI of fibers (n = 100) were evaluated using ImageJ (Supplemental Fig. S.1). Average pore sizes of the membranes fabricated using different solvent ratios (DMF:THF = 10:0, 7:3, and 5:5) were 2 ± 1 μm, 1.7 ± 0.6 μm, and 1.8 ± 0.6 μm, respectively. The average fiber DI of the membranes fabricated using DMF:THF = 5:5 was 5 ± 2 μm. On the other hand, the membrane fabricated using a higher ratio of THF in the mixed solvent (DMF:THF = 3:7) showed the greatest average pore size (2.1 ± 0.7 μm), the smallest average fiber DI (1.1 ± 0.5 μm), and the narrowest distribution of the DI. As the membranes fabricated with a higher content of THF in the mixed solvent (DMF:THF = 3:7) showed the most uniform fibrous porous structure, they were used for further studies on pH-responsive morphology changes, NP release, and *in vitro* cytotoxicity. The viscosity of 20 wt% PEG-HEP-MDI-PG in a mixed solvent

(DMF:THF = 3:7) was 0.306 ± 0.003 Pa.s. Viscosity is one of the important factors for electrospinnability. Polymers need to reach a certain threshold molecular weight as reflected by the viscosity of their solutions to get enough entanglement for fiber formation. The PU solution had a sufficiently high viscosity (0.306 ± 0.003 Pa.s.), thereby allowing it to be stretched into continuous fibers. It is important to note that a higher molecular weight polymer or higher solution concentration will lead to an increase in viscosity and greater fiber DI.

The morphology changes in electrospun porous pH-responsive (DMF:THF = 3:7) and control PU membranes under dry and wet conditions (pH 4.5 and 7.0) were evaluated on the basis of changes in the average pore size, average DI of fibers, and the thickness of the membranes (Fig. 2, Table 1). Pore size, fiber DI, and thickness of membranes were evaluated from micrographs taken using an SEM (magnification: ×1,000) using ImageJ. Pore sizes evaluated from the surface of the electrospun membranes might be different from those of the real situation for NP penetration because the actual membrane would have more layers than those shown in the SEM images. Average pore sizes of porous pH-responsive PU membranes under dry condition, pH 4.5, and pH 7.0 were 2.3 ± 0.7 μm, 1.8 ± 0.6 μm, and 2.2 ± 0.6 μm, respectively (Supplemental Fig. S.2). Average DIs of fibers of porous pH-responsive membranes under dry condition, pH 4.5, and pH 7.0 were 0.9 ± 0.4 μm, 1.4 ± 0.5 μm, and 1.2 ± 0.5 μm, respectively. The difference in the pore sizes of porous pH-responsive PU membranes at pH 4.5 and 7.0 was 0.4 μm; however, the control PU membrane showed a difference of 0.2 μm between pH 4.5 and 7.0. The porous pH-responsive PU membrane had a smaller pore size at pH 4.5 than at pH 7.0 owing to the pH-responsive swelling of electrospun fibers. The fiber DI of the pH-responsive membrane was 0.2 μm larger at pH 4.5 than at pH 7.0. The swollen fiber might occupy space between fibers and reduce pore sizes. The pH-responsive swelling of the fibers

contributes not only to change in pore sizes but also to increased membrane thickness. The thickness of the porous pH-responsive membrane showed noticeable differences at pH 4.5 (50 ± 2 μm) and pH 7.0 (35 ± 1 μm). At pH 4.5, the thickness of the porous pH-responsive membrane was 1.47 times greater than that at pH 7.0 owing to the protonation of pH-sensitive groups.[28] Such pH-responsive morphology changes contributed to pH-responsive PSN permeability as described in the following sections.

Zeta-potentials of control PU (PEG-HD-MDI-HD) and porous pH-responsive PU (PEG-HEP-MDI-PG) membranes (DMF:THF = 3:7) were calculated from the streaming current at pH values ranging from 3.5 to 8.5 (Fig. 3). The control PU membrane showed an isoelectric point at pH 3.9. It showed a desirable zeta-potential profile as a control membrane, with negative values in pH range of 3.9 to 8.5, compared to the porous pH-responsive membrane. The zeta-potential of the control PU membrane was -7.9 mV at pH 4.5 and -25.4 mV at pH 7.0. The negatively charged surface has no attractive electric force, but it has repulsive electric force. However, the porous pH-responsive PU membrane may have strong attractive forces with anionic NPs at pH 4.5 due to the positively charged surface at pH 4.5 (14.2 mV). The isoelectric point of the porous pH-responsive membrane was pH 7.2, and the membrane showed zeta-potential close to zero (2.2 mV) at pH 7.0. The porous pH-responsive PU membrane can have a strong electric attractive force at normal pH of the human vaginal tract (pH 3.5-4.5) and almost no electrostatic force at neutral pH (elevated pH of the vaginal tract resulting from the introduction of seminal fluid during heterosexual intercourse).

The electrospun porous PU membrane can be used as a semi-permeable membrane in reservoir-IVRs for pH-responsive release of NPs as illustrated in Fig. 4. Briefly, a reservoir-IVR can be made of water-swellable non-pH-responsive PU, and the reservoir can be filled with an

NP suspension. The suspension of NPs would be recommended to avoid air bubbles in the reservoir and achieve fast out-diffusion of NPs due to pH change, i.e., fast pH-responsiveness. In addition, a reservoir-IVR with medical grade PUs composed of two parts, i.e., the IVR itself and two capsules made up of resin at opposite sides of the ring, can be fabricated using the electrospun porous PU membranes (Supplemental Fig. S3). Each capsule can be filled with NPs, and the electrospun pH-responsive PU membrane can be used to seal the top of the capsule. The membrane can be secured at the top by another fenestrated resin cap using medical grade glues to achieve the pH-responsive release of the NPs.

The electrospun pH-responsive PU (PEG-HEP-MDI-PG) membrane showed a positively charged surface and a larger DI of electrospun fibers, thicker cross-section, and smaller pore size at pH 4.5 than at pH 7.0. This is because the pH-sensitive molecule HEP (pKa 6.4) on the prepolymer chain of the PU was protonated at pH 4.5 and de-protonated at pH 7.0 [25]. Protonated HEP causes the electrospun fibers to swell more at pH 4.5. The higher swelling ratio leads to the larger DI of fibers, therefore leading to a thicker cross-section and a smaller interfiber pore size. Overall, the "window" membrane (electrospun porous pH-responsive PU membrane) can control the release of the NPs from the reservoir by a pH-responsive change in the electrostatic interaction between loaded NPs and morphologies (pore size and thickness of the membrane).

3.2. pH-responsive nanoparticle release studies using a model nanoparticle (PSNs)

Anti-HIV drugs and siRNAs can be loaded into the nanocarriers through hydrophobic or electrostatic interactions. Commercially available blue-dyed PSNs, the surfaces of which were modified to bear the carboxylic group (COOH), were used for NP release studies as a model nanocarrier. The PSNs could be a model NP of siRNA-loaded nanocarriers with a negatively

charged surface, such as NPs, fabricated using PLGA-PEG-COOH and PVA by a double-emulsion solvent evaporation method [17,47–49]. Moreover, PSNs have been used as a model nanocarrier for *in vitro* mucus penetration [50] and cellular uptake [51–54] studies. The PSNs showed narrow polydispersity in VFS (0.23 ± 0.02 and 0.04 ± 0.01 at pH 4.5 and 7.0, respectively) and could be observed with the naked eye. The average particle size and zeta-potential of PSNs were measured at pH 4.5 and 7.0 (Table 2). These PSNs showed a minor difference in average zeta-potential and hydrodynamic particle size, at pH 4.5 (-16 ± 3 mV, 174 ± 1 nm) and at pH 7.0 (-20 ± 4 mV, 215 ± 2 nm), due to the deprotonation of the carboxylic groups at pH 7.0.[55]

In vitro PSN release studies were performed using porous pH-responsive (PEG-HEP-MDI-PG) (DMF:THF = 3:7) and control (PEG-HD-MDI-HD) PU membranes (Fig. 5). The pH-responsive PSN permeability of the porous pH-responsive PU membrane is distinguished from PSN release profile of the control PU membrane (Fig. 5A, 5B). These porous pH-responsive membranes revealed close-to-zero release of PSNs at pH 4.5; however, they showed sustained release of PSNs at pH 7.0 (Fig. 5B, 5C). At pH 7.0, 60 ± 6% of the feed amount of PSNs (0.8 mL of 1 mg/mL PSNs) was released after 24 h. There was a rapid release of PSNs at pH 7.0 for 3 h (51 ± 5%) driven by the relatively larger difference in concentration between the upper and bottom chambers of Franz cells. Such pH-responsive release of anionic NPs has two principal mechanisms. First, changes in the electrostatic interaction between the NPs and the electrospun porous pH-responsive membranes at pH 4.5 and pH 7.0 could lead to the pH-responsive NP release. The zeta-potential of the porous pH-responsive membrane was positive (14.2 mV) at pH 4.5 and close-to-zero at pH 7.0 (2.2 mV) because the conjugate acid of the pH-sensitive moiety piperazine has a pKa of 6.5. The porous pH-responsive membrane had an electrostatic

interaction with anionic PSNs at acidic pH (pH 4.5) but almost no electrostatic interaction at neutral pH. Second, morphology changes in the membrane at pH 4.5 and pH 7.0 contributed to the pH-responsive release of NPs. The porous pH-responsive PU membrane was thicker at pH 4.5 (50 ± 2 μm) than at pH 7.0 (34 ± 1 μm), with a smaller pore size at pH 4.5 (1.8 ± 0.6 μm) than at pH 7.0 (2.2 ± 0.6 μm) due to the swelling of the electrospun pH-responsive PU fibers.

The intravaginal pH change by seminal fluid occurs immediately after the ejaculation of semen [20–22]. According to Tevi-Bénissan et al., full vaginal elimination of semen takes 20 h, and the vaginal ecological microenvironment returns to its normal acidic pH 48 h after intercourse [20]. It is inferred that the elevated pH could be maintained around neutral for at least a couple of hours because the sperm needs an alkaline or neutral environment to be able to survive and swim to fertilize the egg [23,24]. The pH-responsive PU membranes allowed the rapid release of PSNs at pH 7.0 for 2 h (30 ± 4%). This result reveals that the pH-responsive PU membranes have a potential use serving as a “window” membrane of IVRs for the on-demand release of nanocarriers. Additionally, the PSN release study was performed at pH 5.5 as an intermediate pH of 4.5 and 7.0 for further assessment of the pH-responsive release. The results of PSN release studies at pH 5.5 showed around a middle point between pH 4.5 and 7.0 in the case of the control PU membrane (52 ± 2% for 24 h). For the pH-responsive PU membrane, increasing pH from 4.5 to 5.5 allowed PSNs to start to pass through the membrane. However, the 24 h release percentage was 22 ± 2%, which was only one-third of the release at pH 7.0 (60 ± 6%). This result correlates well with the zeta-potential of the membrane. At pH 5.5, the pH-responsive PU membrane was still positively charged as reflected by its zeta-potential of 10.5 mV at pH 5.5 (closer to 14.2 mV at pH 4.5 than 2.2 mV at pH 7.0) and exerted an attractive electric force on PSNs, thereby restricting their release across the membranes. On the other hand,

the cumulative amount of the PSNs released from the control membranes was found to be 32 ± 6% at pH 4.5, 52 ± 2% at pH 5.5, and 67 ± 5% at pH 7.0 for 24 h. According to these results, the control membrane did not show the desired pH-responsive on-demand release of PSNs.

To better explain the pH-responsive release of PSNs, the association between the electrospun PU (porous pH-responsive and control) membrane and the PSN was evaluated (Fig. 6). Round membrane samples (DI: 0.8 cm) were prepared and immersed in 6.4 mL of 0.125 mg/mL PSN suspension at 37 °C with gentle horizontal shaking (100 rpm). These PSNs showed a relatively constant difference (12 ± 3%) in the number associated with the porous pH-responsive membrane between pH 4.5 and pH 7.0 for 24 h. Such difference resulted from changes in the electrostatic interaction between porous pH-responsive membrane and PSNs, as well as changes in the morphology of the membrane. Although the average pore size at pH 4.5 (1.8 ± 0.6 μm) was smaller than that at pH 7.0 (2.2 ± 0.6 μm), the strong electric attractive force at pH 4.5 might have caused a higher amount of PSN to be associated with the porous pH-responsive membrane than that at pH 7.0. The control membrane showed the lowest amount of associated PSN at pH 7.0 because of the strong repulsion force between the negatively charged control membrane surface (-25.4 mV) and PSNs (-20 ± 4 mV). The repulsion force at pH 7.0 might have hindered the penetration of PSNs through the control PU membrane at an early stage of penetration by preventing PSNs from approaching close to the membrane surface.

To further assess the pH-responsive release of NPs, release profiles of PSNs were recalculated using the following equation:

$$a\ (\%) = \frac{b}{c - d} \times 100$$

where a is the % of released PSNs at each time point, b is the released amount of PSNs (μg) in the bottom chamber, c is the feed amount of PSNs to the upper chamber (μg), and d is the

amount of PSNs associated with the membrane (μg). Deducting the amount of adsorbed NPs allows us to take a close look at the diffusion of PSNs in their free unbound form. After the deduction, there was a continuous release of free PSNs at pH 7.0 for 24 h. However, there was close-to-zero release at pH 4.5 (4 ± 4%) for 24 h through the porous pH-responsive PU (PEG-HEP-MDI-PG) membrane (Fig. 7). The control PU membrane showed a minor difference in the cumulative release of PSNs (36.7% at 24 h) compared to porous pH-responsive PU membrane. The difference in the release of PSNs between pH 4.5 and pH 7.0 through the control PU membrane could be due to the difference in the surface charge of the control PU at pH 4.5 and 7.0. The control PU has a much more negative zeta-potential at pH 7.0 (-25.4 mV) than at pH 4.5 (-7.9 mV) as shown in Figure 3. A much stronger Coulomb repulsion exists between PSNs and the control PU membrane at pH 7.0 than at pH 4.5 and contributes to accelerated NP penetration through the membrane.

3.3. pH-responsive nanoparticle release studies using CCR5 siRNA-encapsulated SLNs

To further demonstrate the utility of the electrospun pH-responsive PU membrane (PEG-HEP-MDI-PG) in achieving pH-responsive intravaginal delivery of nanomedicines, CCR5 siRNA-encapsulated-SLN was prepared by the emulsion solvent diffusion method and used for pH-responsive release study. The prepared CCR5 siRNA-encapsulated SLNs had a mean size of 267 ± 32 nm, with a zeta potential of -19 ± 5 mV at 5 μg/mL in distilled water. The siRNA encapsulation efficiency was 67.6%, which was determined using the formula EE% = [{amount of total siRNA (μg)-amount of unencapsulated siRNA (μg)} / {amount of total siRNA (μg)}] × 100

The electrospun pH-responsive PU membrane showed a significantly higher release of CCR5 siRNA-encapsulated SLNs at pH 7.0 (59 ± 8%) than at pH 4.5 (28 ± 4%) after 24 h of

incubation (Fig. 8). This pH-responsive release can also be explained by the difference in electrostatic interaction between the membrane and CCR5 siRNA-encapsulated SLNs and morphologies of the membrane at pH 4.5 and pH 7.0. Compared to the release of PSNs, CCR5 siRNA-encapsulated SLNs presented a burst release of 21 ± 4% (1 h) at pH 4.5 instead of close-to-zero release. Moreover, SLNs were released much faster (51 ± 5%) than PSNs (4 ± 1%) at pH 7.0 for 1 h. This result reveals that the CCR5 siRNA-encapsulated SLNs has much higher penetration efficiency through the membrane than PSNs, which may be due to the difference in physicochemical characteristics such as hydroxyl groups on SLN and carboxylic groups on PSNs.

Again, the electrospun pH-responsive membrane demonstrated their potential use in the smart intravaginal release of nanomedicines, although further optimization is necessary to achieve pH-responsive on-demand release (no release at the normal acidic pH of the vaginal tract and triggered release at elevated pH) of the therapeutic NP-CCR5 siRNA-encapsulated SLN.

3.4. In vitro cytotoxicity studies of the electrospun pH-responsive PU (PEG-HEP-MDI-PG) membrane

The human vaginal epithelial cell line "VK2/E6E7" and the human T-cell line "Sup-T1" were used for an *in vitro* cytotoxicity assay of electrospun porous pH-responsive PU membranes considering their potential application as "window" membranes in reservoir-IVRs for the pH-responsive release of NPs, which may serve as nanocarriers for anti-HIV drugs and siRNAs. Electrospun porous pH-responsive PU (PEG-HEP-MDI-PG) membrane elution medium that was used to treat the cells did not present a cytotoxicity effect compared to the positive control (Fig. 9). We also evaluated the production of pro-inflammatory cytokines (IL-6, IL-8, and IL-1β) after treating the cells with the elution medium, and the obtained results are shown in Fig. 10. Compared to the positive controls induced by the LPS and N-9, the PU membrane eluent did not generate significant changes in the expression of the pro-inflammatory markers, suggesting that the membranes did not induce an inflammatory microenvironment.

4. Conclusion

The pH-responsive interconnected porous PU membrane was fabricated by electrospinning using a pH-responsive PU (PEG-HEP-MDI-PG) copolymer. The porous pH-

responsive membranes showed pH-dependent changes in morphology (pore sizes, fiber DIs, and thickness) and surface charge. Such porous pH-responsive PU membranes demonstrated pH-dependent release of color-dyed PSNs (-COOH) and CCR5 siRNA-encapsulated SLNs. There was close-to-zero release of PSNs in VFS at pH 4.5 (normal pH of the female genital tract). At pH 7.0, the release of PSNs significantly increased to 60 ± 6% in VFS at neutral pH (elevated pH of the female genital tract). Similarly, penetration of CCR5 siRNA-encapsulated SLNs through the PEG-HEP-MDI-PG membrane was more than twofold as much at pH 7.0 as at pH 4.5. The electrospun porous pH-responsive PU membrane showed no noticeable toxicity to human vaginal epithelial cell line (VK2/E6E7) and human T-cell line (Sup-T1). In addition, the elution medium collected from the membranes did not induce an inflammatory microenvironment. Overall, the newly electrospun porous pH-responsive PU membrane demonstrated potential use as a novel biomaterial for "smart" intravaginal delivery of therapeutic NPs.

Acknowledgments

This research was funded in part by the Natural Science and Engineering Research Council of Canada (NSERC) Discovery Grant awarded to Dr. Song Liu (Grant No.: RGPIN/04922-2014) and an NSERC Discovery Grant (Grant No.: RGPIN-2015-06008) awarded to Dr. Emmanuel Ho.

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Figure legends

Fig. 1 SEM images of electrospun pH-responsive PU (PEG-HEP-MDI-PG) membranes at a voltage of 30 kV and PU concentration of 20 wt%. The volume ratios of DMF:THF were (A) 10:0, (B) 7:3, (C) 5:5, and (D) 3:7. Scale bars are shown in the images.

Fig. 2 Morphology of electrospun porous pH-responsive PU (PEG-HEP-MDI-PG) membranes (DMF:THF = 3:7): dry (A), pH 4.5 (B), pH 7.0 (C), and control PU (PEG-HD-MDI-HD)

membranes: dry (D), pH 4.5 (E), pH 7.0 (F). Scale bars are shown in the images.

Fig. 3 Influence of streaming pH on the zeta-potential of the electrospun PU membranes at pH ranging from 3.5 to 8.5. (A) Control PU (PEG-HD-MDI-HD) membrane and (B) porous pH-responsive PU (PEG-HEP-MDI-PG) membrane. Data are expressed as mean ± SD; $n = 3$. Zeta-potential was calculated using the Helmholtz–Smoluchowski equation.

Fig. 4 Diagram of the proposed use of the electrospun porous pH-responsive PU membrane as a "window" membrane in reservoir-IVR for controlled release of anionic nanoparticles release: (A) IVR; (B) window membrane; (C) reservoir. pH-responsive change in electrostatic interaction between the pH-responsive membranes and the anionic nanoparticles and morphology of the membrane contribute to the smart release of nanoparticles.

Fig. 5 *In vitro* nanoparticle permeation studies of (A) control PU (PEG-HD-MDI-HD) membrane, (B) porous pH-responsive PU (PEG-HEP-MDI-PG) membrane, and (C) photo of the study using porous pH-responsive PU (PEG-HEP-MDI-PG) membrane by Franz cells. Cumulative release of the nanoparticle in percentage (%) for 24 h was evaluated at pH 4.5, pH 5.5, and pH 7.0. Anionic blue-dyed nanoparticles (PSNs, 200 nm) were used. Temperature was maintained at 37°C. Data are expressed as mean ± SD; $n = 3$.

Fig. 6 Association of PSNs on electrospun control and porous pH-responsive PU membranes at pH 4.5, pH 5.5, and pH 7.0. Data are expressed as mean ± SD; $n = 3$.

Fig. 7 *In vitro* nanoparticle release profiles after deduction of the associated PSNs with membrane (A) control PU (PEG-HD-MDI-HD) membrane, (B) porous pH-responsive PU (PEG-HEP-MDI-PG) membrane. Cumulative release of the nanoparticle in percentage (%) for 24 h evaluated at pH 4.5, pH 5.5, and pH 7.0. Data are expressed as mean ± SD; $n = 3$.

Fig. 8 *In vitro* permeation studies of CCR5 siRNA-encapsulated SLNs through porous pH-

responsive PU (PEG-HEP-MDI-PG) membrane in VFS. Cumulative release of the CCR5 siRNA-loaded SLNs in percentage (%) for 24 h was evaluated at pH 4.5 and pH 7.0. Temperature was maintained at 37°C. Data are expressed as mean ± SD; $n = 3$.

Fig. 9 *In vitro* biocompatibility of porous pH-responsive PU (PEG-HEP-MDI-PG) membrane tested with VK2/E6E7 and Sup-T1 cells. MTS assay was conducted for analyzing cell viability. Data are normalized to the negative control and expressed as mean ± SD; $n = 3$. One-way analysis of variance was performed for all results, with $p < 0.05$ considered as significant. Negative control includes the cells cultured in the medium only. To induce cell death in positive control, 1 M acrylamide dissolved in regular cell culture medium was used. N: negative control, P: positive control.

Fig. 10 Impact of porous pH-responsive PU (PEG-HEP-MDI-PG) membranes on proinflammatory cytokine production (A) Interleukin IL-1β, (B) IL-6, and (C) IL-8 production. Negative control was a plain medium, and positive control was 200 µg/mL of nonoxynol-9 or 50 µg/mL of lipopolysaccharide-treated cells for IL-1β and IL-6/IL-8, respectively. Data are expressed as mean ± SD; $n = 3$. One-way analysis of variance was performed for all results, with $p < 0.05$ considered as significant. N: negative control, P: positive control.

Table 1. Average pore size and diameter of electrospun porous pH-responsive (PEG-HEP-MDI-PG) and control (PEG-HD-MDI-HD) PU membranes under dry and wet conditions in VFS at pH 4.5 and 7.0.

		Dry	pH 4.5	pH 7.0
pH-responsive (PEG-HEP-MDI-PG)	Average pore size (µm)	2.3 ± 0.7	1.8 ± 0.6	2.2 ± 0.6
	Average diameter of fibers (µm)	0.9 ± 0.4	1.4 ± 0.5	1.2 ± 0.5
	Average thickness (µm)	28.6 ± 0.7	50 ± 2	35 ± 1
Control (PEG-HD-MDI-PG)	Average pore size (µm)	2.0 ± 0.6	1.5 ± 0.4	1.7 ± 0.4
	Average diameter of fibers (µm)	0.7 ± 0.2	0.9 ± 0.3	0.8 ± 0.2

Average thickness (μm)	22.9 ± 0.8	29 ± 2	25.0 ± 0.9

Table 2. Physicochemical characteristics of blue-dyed polystyrene nanoparticles (PSNs) (data are expressed as mean ± SD; n = 3).

	pH 4.5	pH 7.0
Particle size (nm, 0.1 μg/mL)	174 ± 1	215 ± 2
Zeta potential (mV, 0.1 μg/mL)	-16 ± 3	-20 ± 4

Design and development of pH-responsive polyurethane membranes for intravaginal release of nanomedicines

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Statement of Significance

Stimuli-responsive intravaginal nanoparticle release is achieved for the first time through new electrospun pH-responsive polyurethane (PU) semi-permeable membranes, which serve as a

"window" membrane of the reservoir-type intravaginal ring (IVR) for the prevention of human immunodeficiency virus (HIV) transmission. Almost no release of nanoparticles was observed at normal pH in the female genital tract (in vaginal fluid simulant [VFS], at pH 4.5); however, a continuous release of nanoparticles was observed at elevated pH in the female genital tract (in VFS, at pH 7.0). This pH-responsive intravaginal release can reduce side effect and drug resistance by avoiding unnecessary exposure. The PU semi-permeable membrane demonstrated potential use as biomaterials for "smart" intravaginal nanoparticle release and has great potential to protect women from HIV.

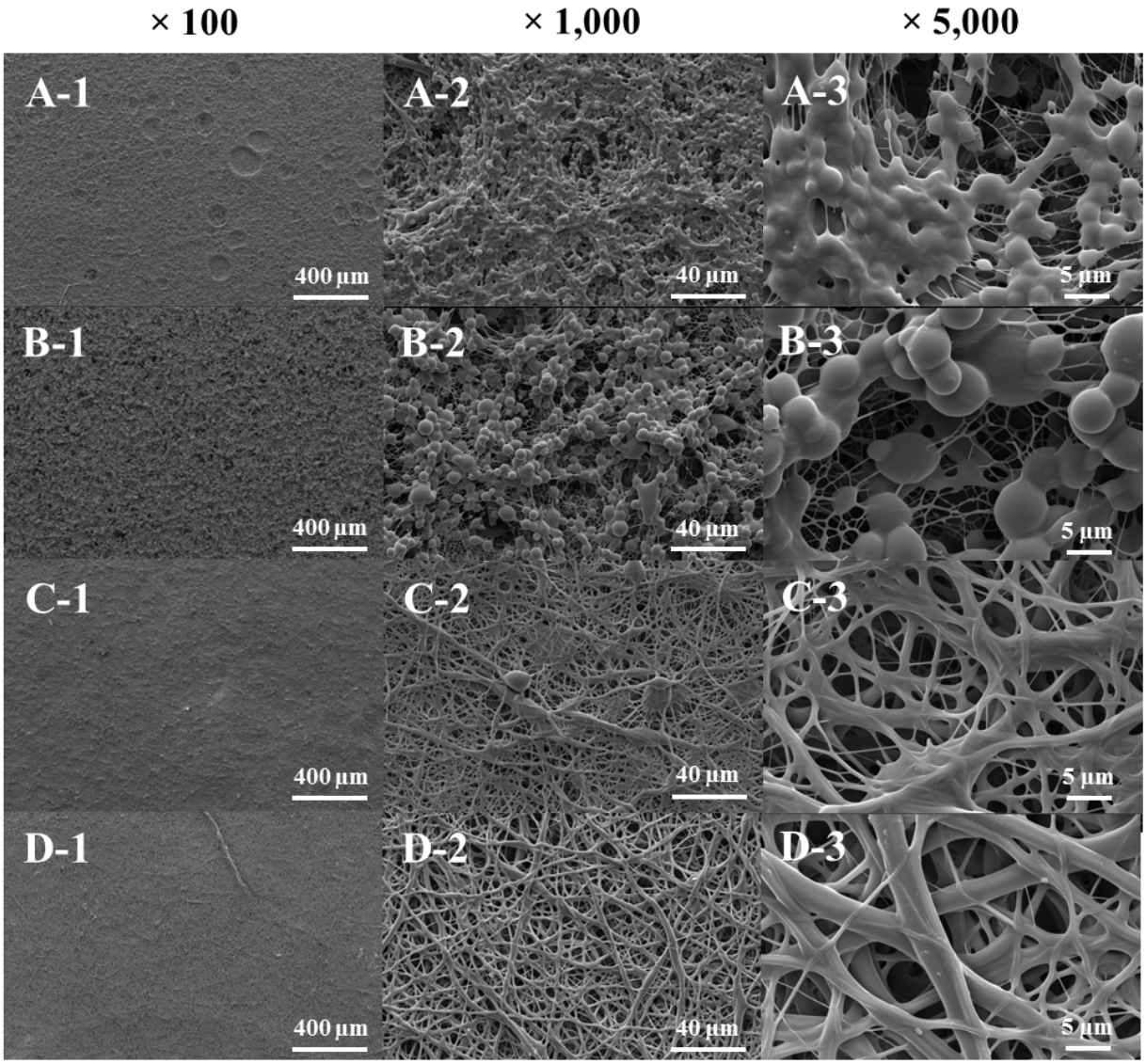


Fig. 1 SEM images of electrospun pH-responsive PU (PEG-HEP-MDI-PG) membranes at a voltage of 30 kV and PU concentration of 20 wt%. The volume ratios of DMF:THF were (A) 10:0, (B) 7:3, (C) 5:5, and (D) 3:7. Scale bars are shown on the images.

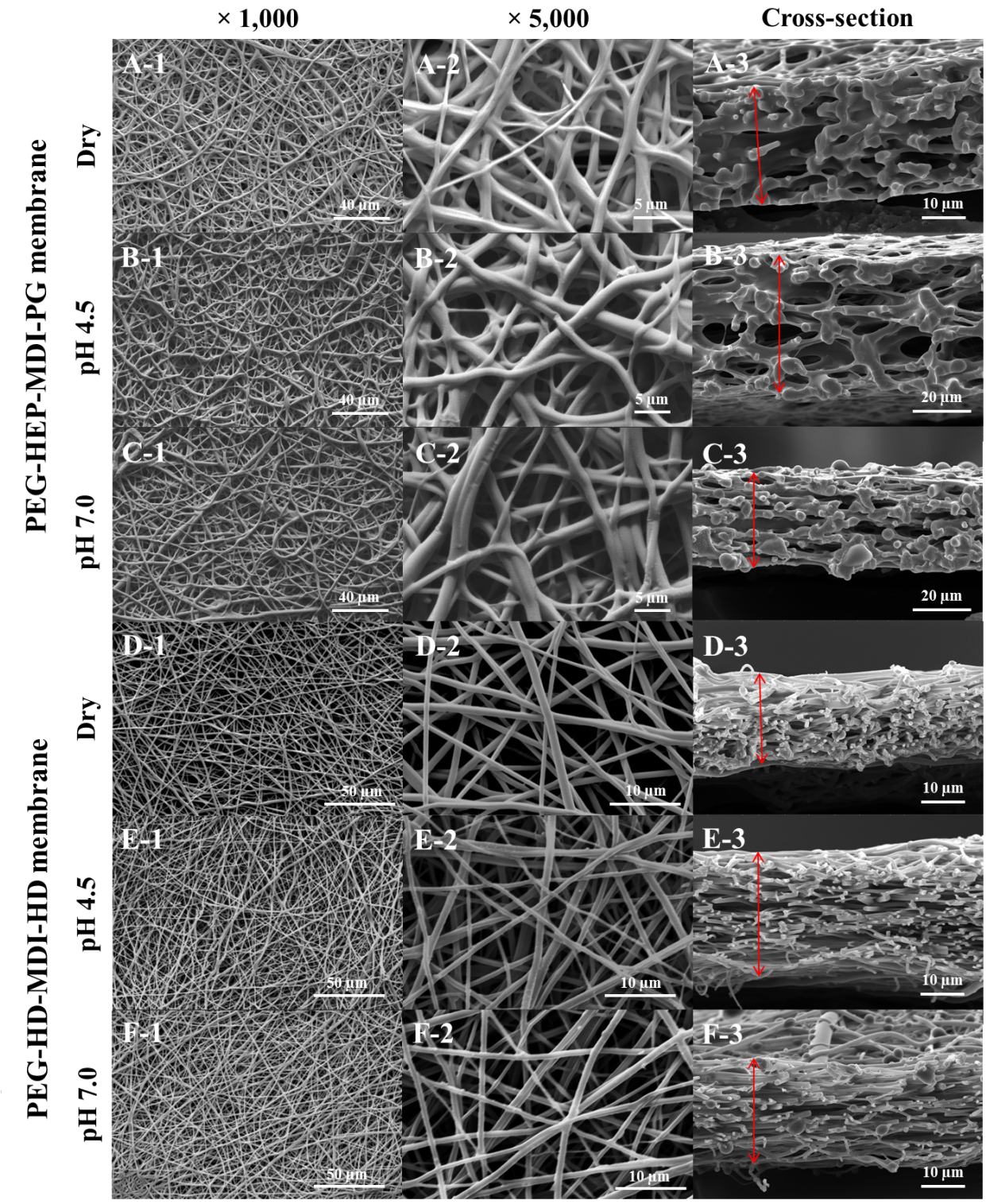
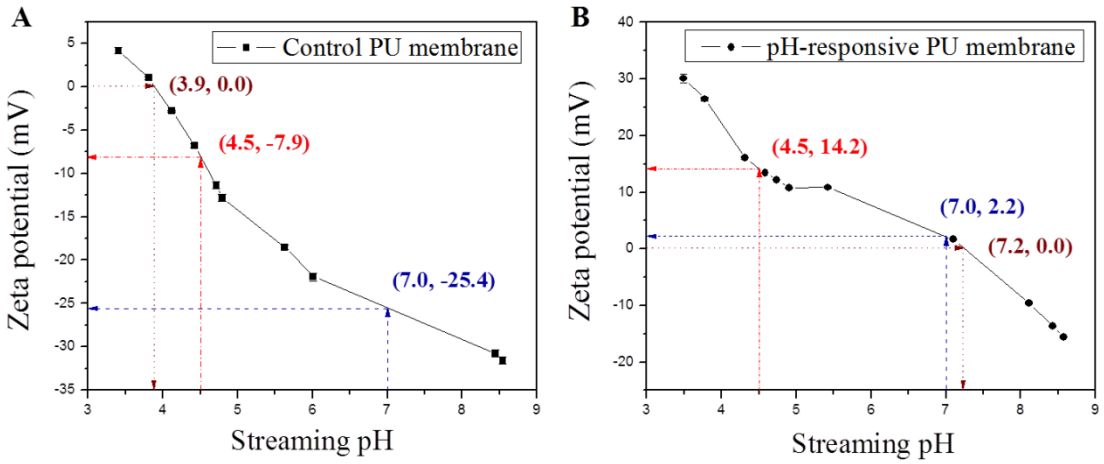


Fig. 2 Morphology of electrospun porous pH-responsive PU (PEG-HEP-MDI-PG) membranes (DMF:THF = 3:7): dry (A), pH 4.5 (B), pH 7.0 (C), and control PU (PEG-HD-MDI-HD) membranes: dry (D), pH 4.5 (E), pH 7.0 (F). Scale bars are shown on the images.



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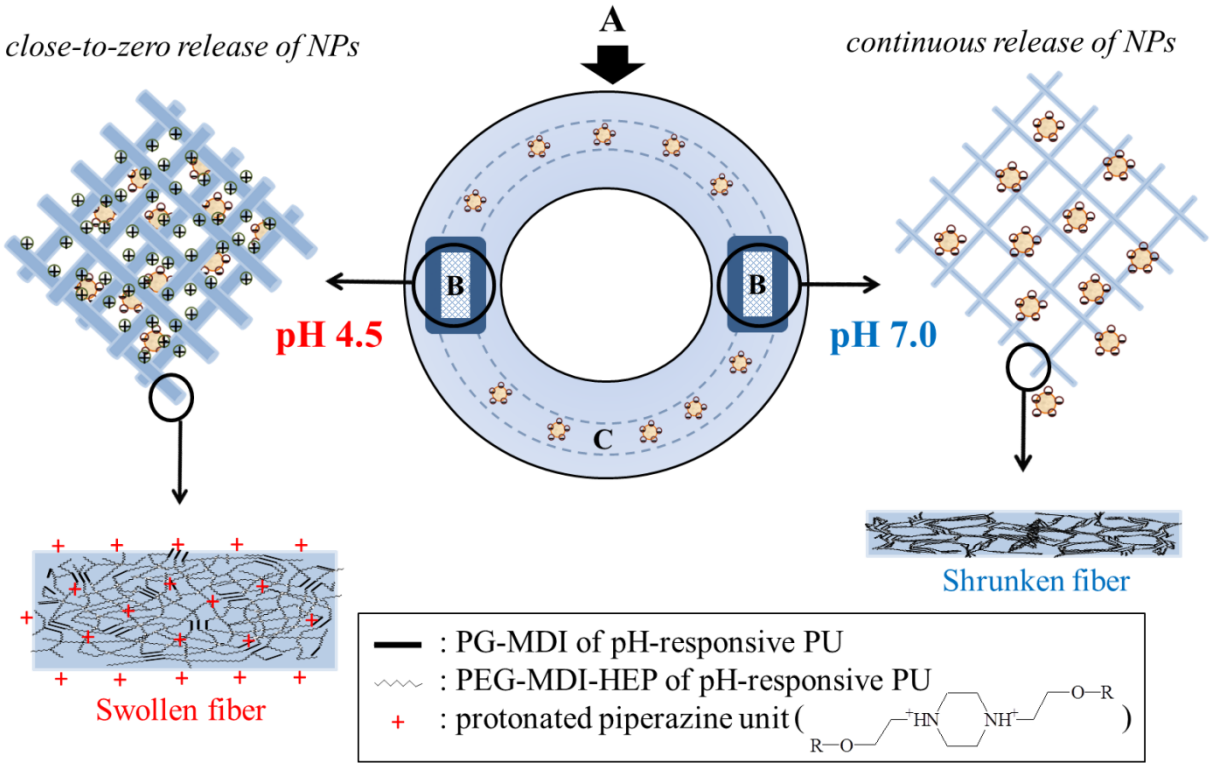

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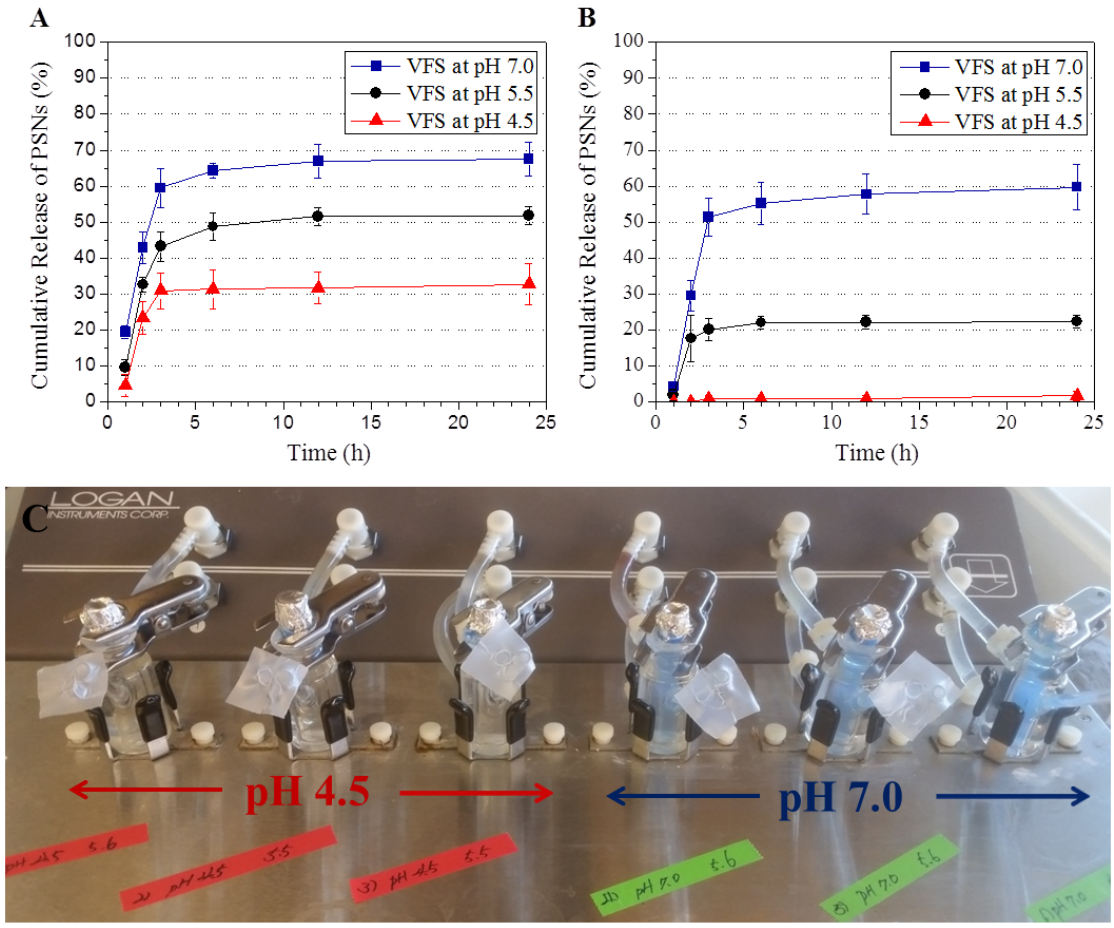

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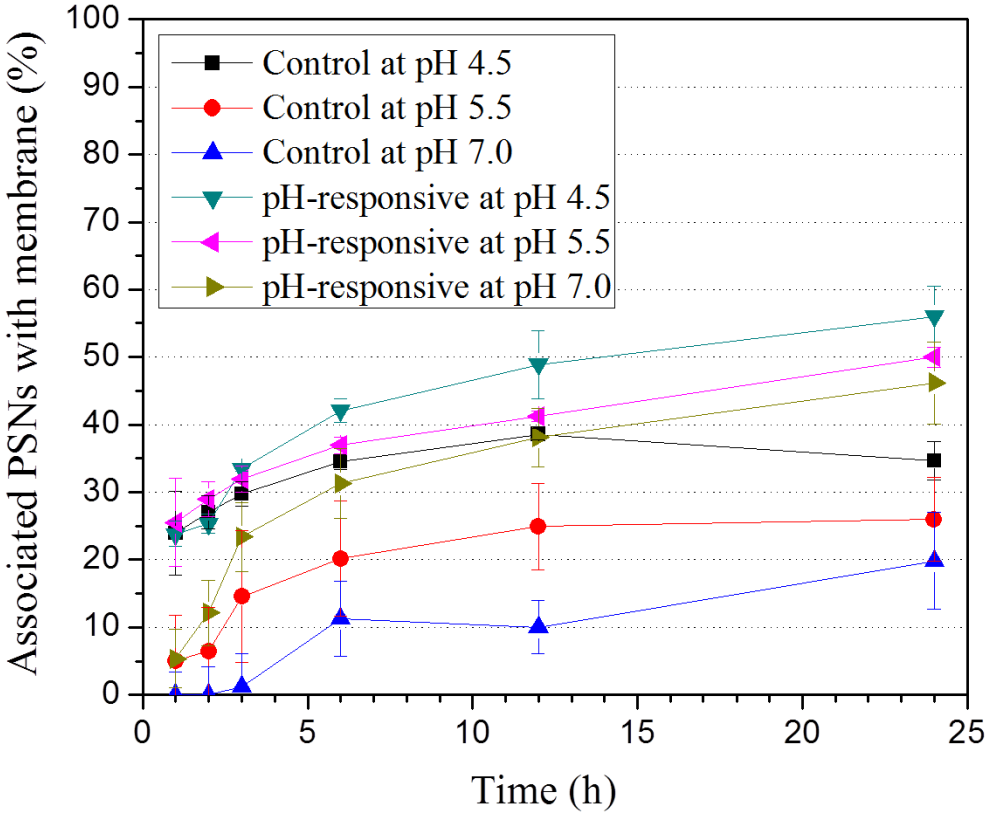

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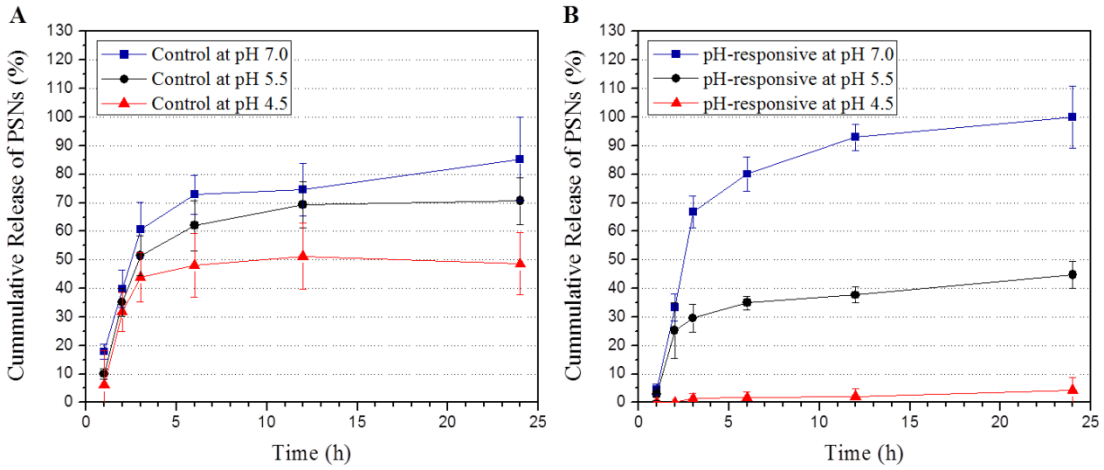

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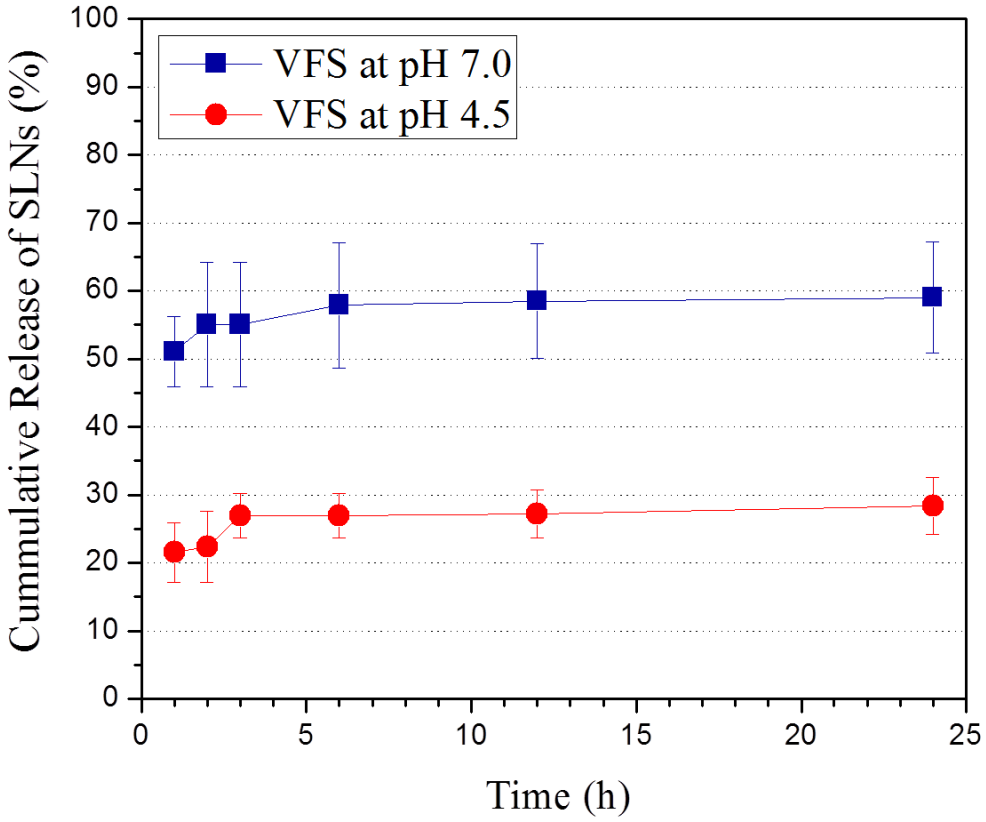

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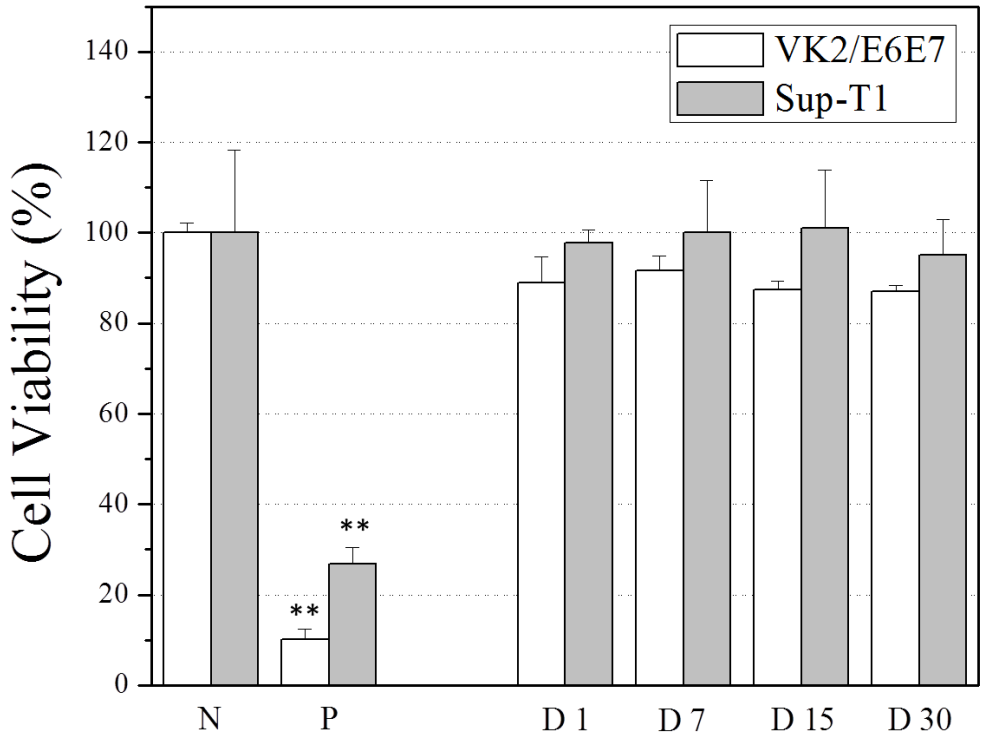

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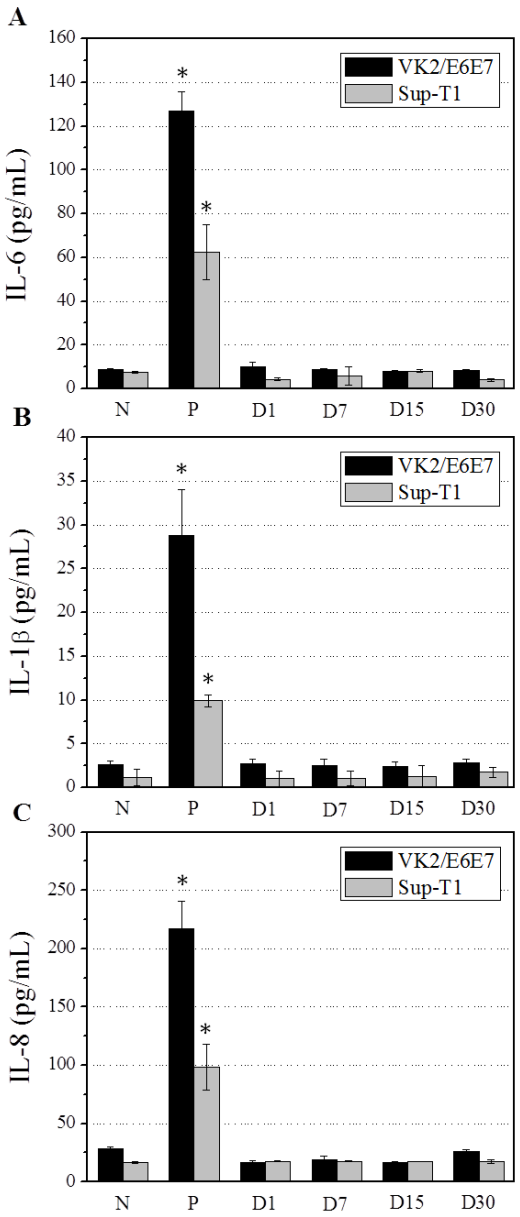

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