화학공학소재연구정보센터

IP(Information Provider) 연구분야 보고서

Bio 분야에서의 Polyurethane의 응용

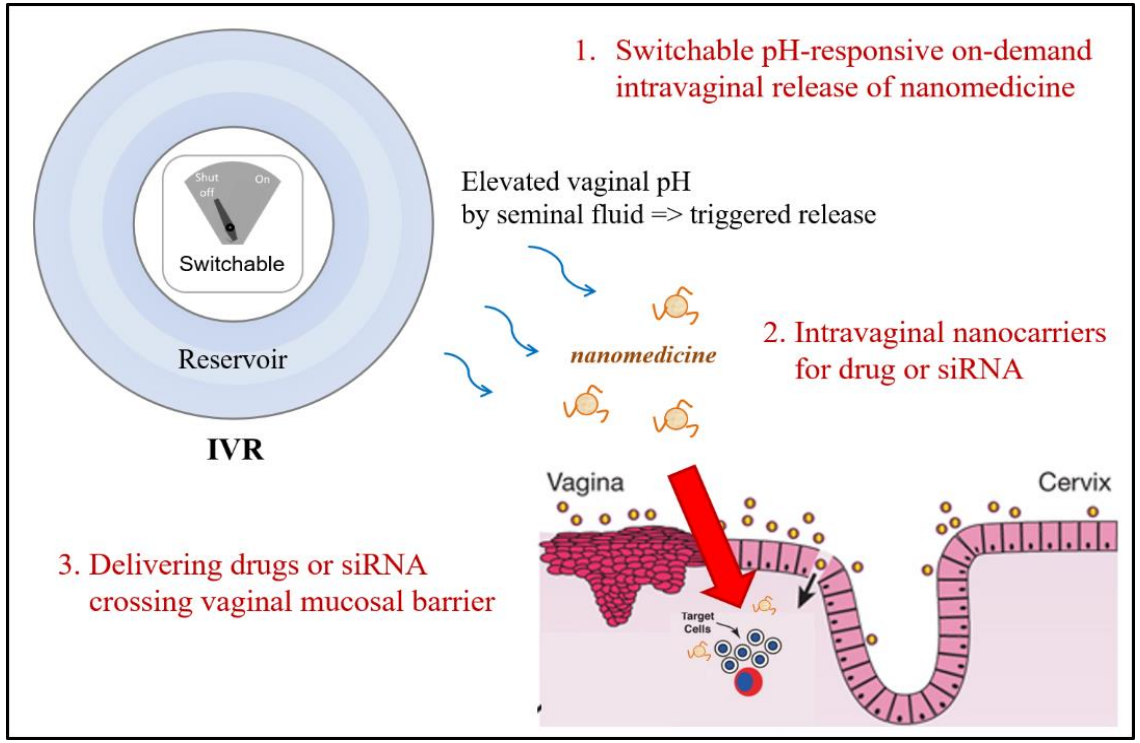
5장. 폴리우레탄의 약물전달에서의 응용

• Polyurethanes in Drug Delivery System (DDS)-Intravaginal delivery of anti-HIV

: 약물전달 (drug delivery system)에서 폴리우레탄은 나노약물전달체 (nanocarrier), 하이드로젤 (hydrogel), 약물 전달 스케폴드 (scaffold) 등의 제조에 사용되어진다. 폴리우레탄은 물리화학적 개질 (physicochemical modification)과 성형 방법 (advanced fabrication tools including 3D printing)의 응용을 통하여, 인체의 다양한 부위와 질병에 적용되어 전달하는 의약물질의 효능을 최대화 하는 동시에 부작용은 최소화하는데 기여하고 있다.

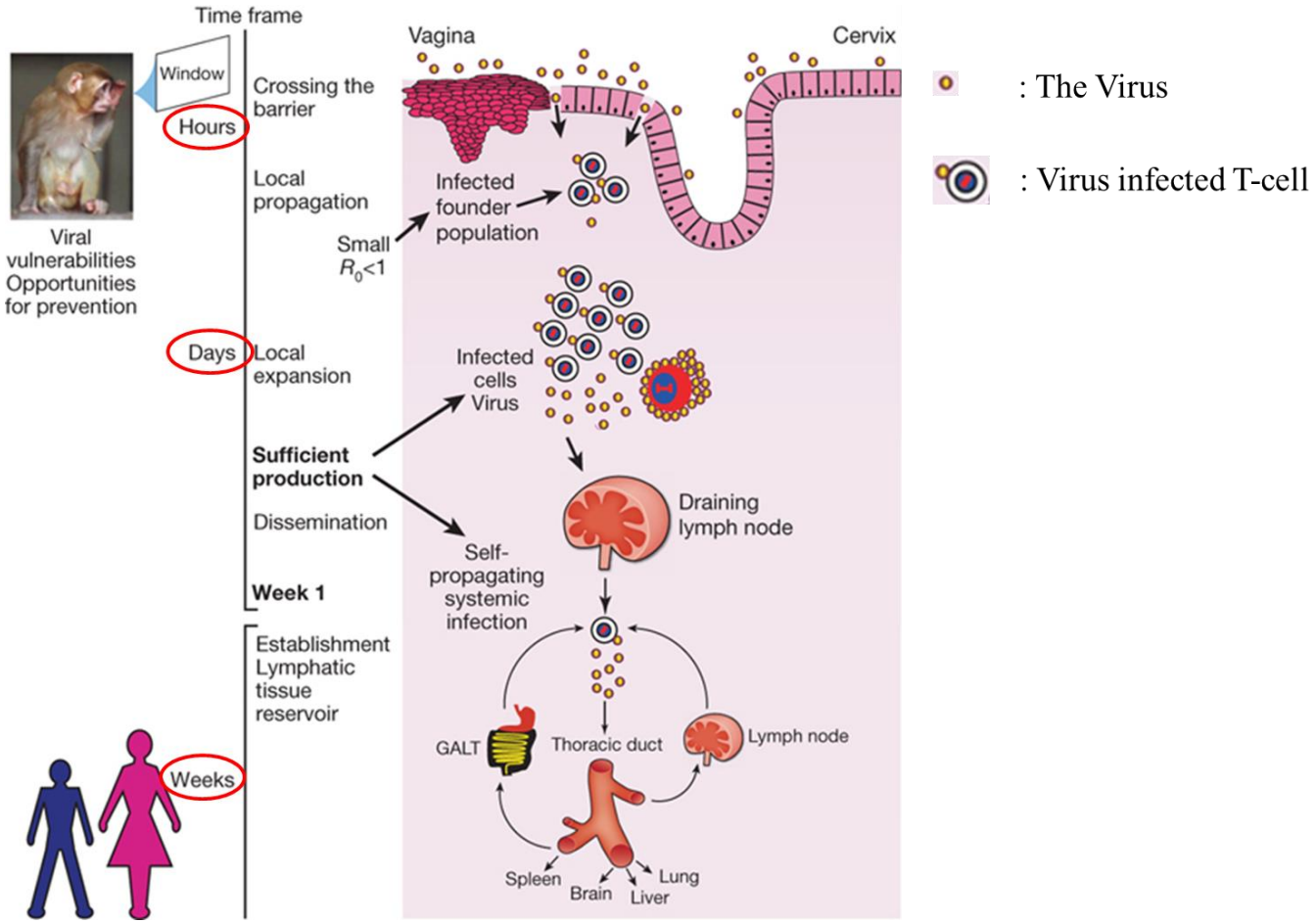
본 보고서에서는 자극 감응성 폴리우레탄을 이용한 "smart" intravaginal delivery of anti-HIV and nanocarrier에 관한 최신 연구를 소개 하고자 한다.

Figure 1. Diagram of "smart" intravaginal delivery system



• 인간 면역 결핍 바이러스 (human immunodeficiency virus, HIV)

Figure 2. Time frame of HIV



: HIV는 여성 유행 전염병으로 분류 되기도 하며, 주로 양성 성행위를 통하여 전염된다.
현재까지 HIV를 감염된 환자에게서 완전히 제거하는 치료제는 없으며, anti-HIV를 통하여 인체내 전이를 억제하고 후천성 면역 결핍 증후군 (Acquired immunodeficiency syndrome, AIDS)로의 진행을 막는다.

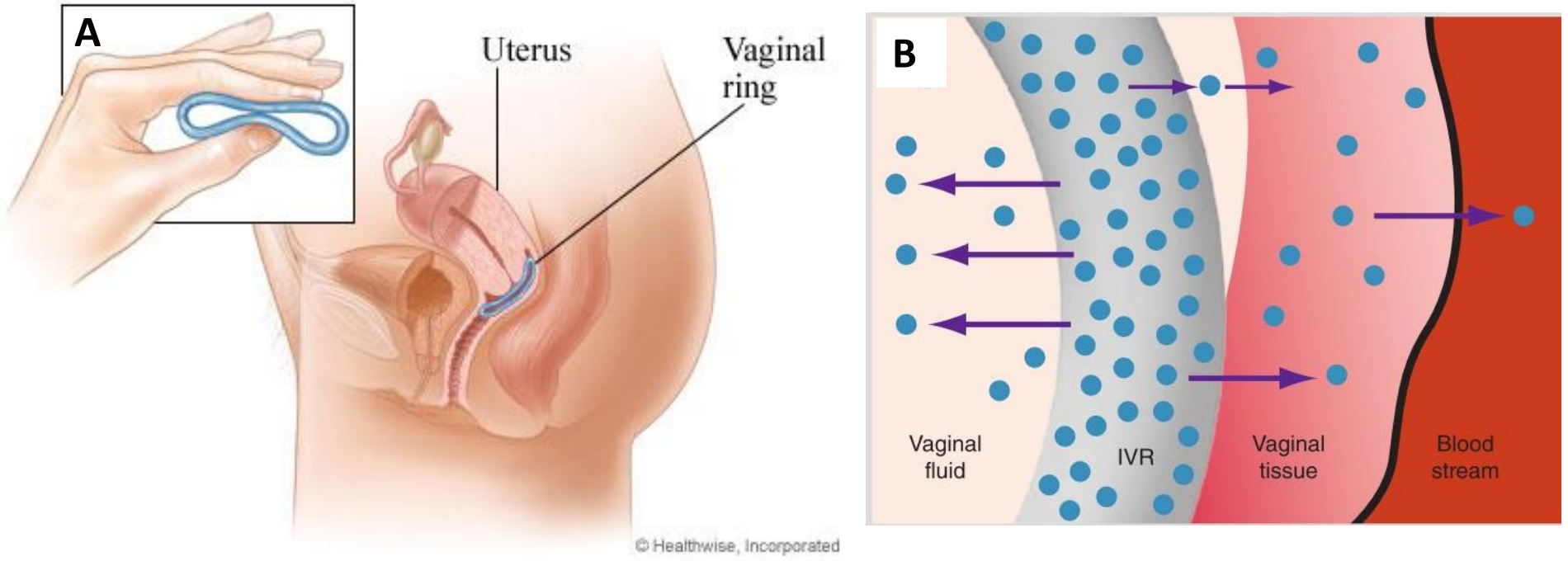
HIV가 성행위를 통하여 질내 감염되면, 수 시간내에 질 점막과 조직을 통과하여, 질 내부의 면역세포에 침입한다. 감염된 면역 세포는 HIV의 생산에 이용되어지며, 수일 이내에 다른 장기로 전이에 충분한 HIV가 인체내에서 만들어진다. 이 HIV 는 초기 감염 후 수주 이내에 인체내의 주요 장기로 전이 되어지므로, 감염 초기의 진단과 치료가 환자의 치료와 다른 사람으로의 전파 방지에 중요한 요소이다 .

Nature, 464, 7286: 217-223, 2010

• Intravaginal ring (IVR)

: HIV의 주요 감염 경로가 질을 통한 인체 감염이므로, IVR을 통한 aniti-HIV의 질내 전달은 초기 감염에 대처 할 수 있는 약물 전달 시스템으로서 장점을 지닌다.
IVR은 남성의 동의 없이도 여성이 사용 할 수 있어, 감염 예방과 치료에 큰 도움이 된다.

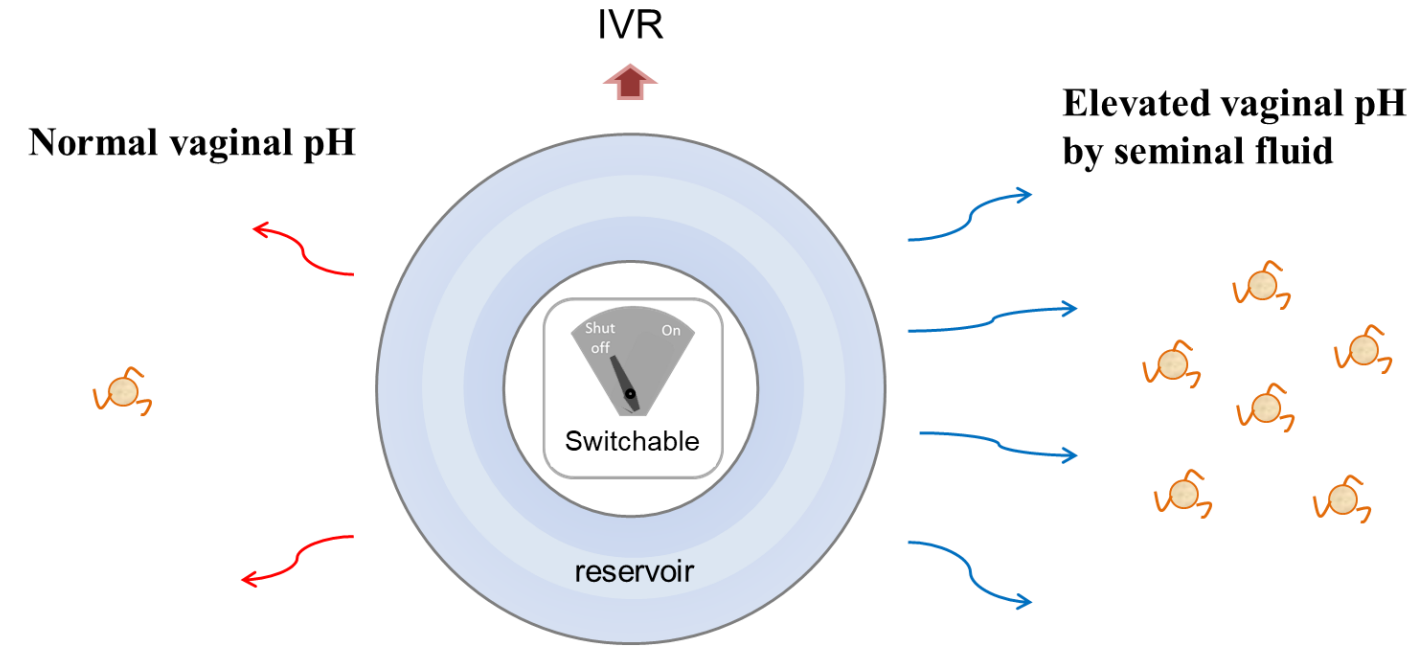
Figure 3. (A) positioning IVR, (B) diffusive transport of drug from an intravaginal ring directly into vaginal tissue or first into a thin conducting layer of vaginal fluid and then into vaginal tissue, with transport into the blood being the ultimate sink.



• pH-responsive intravaginal release of anti-HIV and nanocarrier

: 건강한 여성의 질내 pH는 약산성 (pH 3.5-4.5) 이며, 성 행위시 남성의 정액에 의하여 이 pH가 6.0-7.0으로 상승 한다.
이러한 pH의 변화를 이용하여 IVR에 담지 되어진 anti-HIV 또는 anti-HIV 나노 약물전달체의 “on-demand” release가 가능하다.

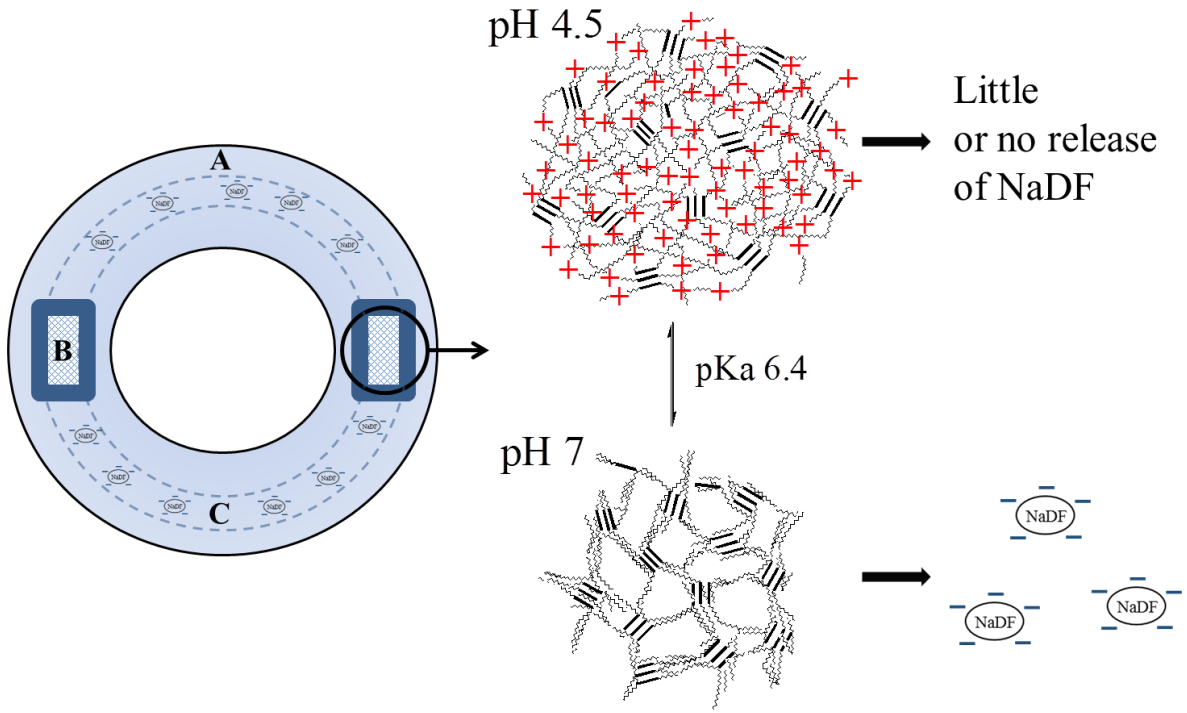
Figure 4. Schematic representation of pH-responsive release of anti-HIV



- **Reversibly pH-responsive polyurethane membranes for on-demand intravaginal drug delivery**

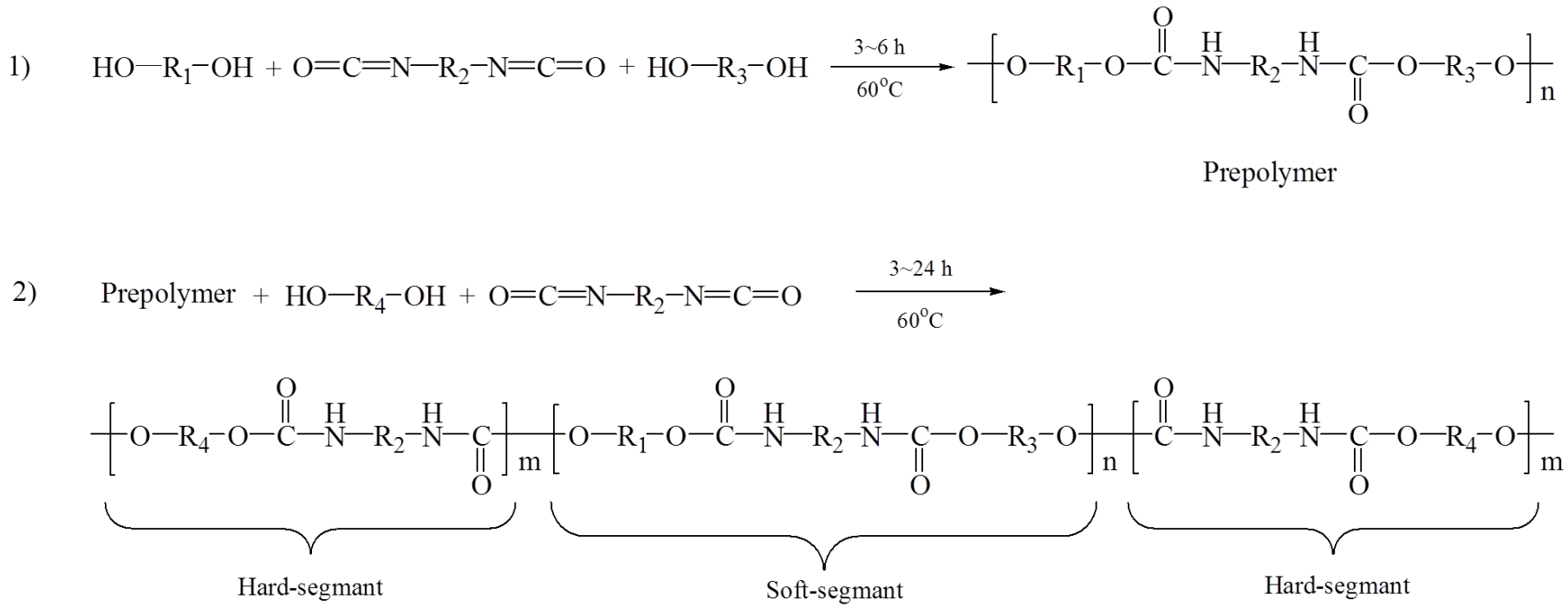
; 본 연구에서는 신규 pH 민감성 폴리우레탄을 합성하고, 이를 IVR의 “window” membrane으로 제작하여, pH 변화에 따른 anionic anti-HIV인 betulinic acid과 bevirimat의 model drug인 diclofenac sodium (NaDF)의 “on-demand” permeability를 확인하였다.

Figure 5. Diagram of proposed use of pH-responsive PU membrane as a window membrane of reservoir-type IVR for controlled on-demand drug release. (A) reservoir-type IVR made of a non-permeable polymer (B) holes for drug loading which are covered by pH-responsive PU membrane (“window membrane“). (C) NaDF loaded in the hollow lumen of the reservoir-type IVR.



- **Reversibly pH-responsive polyurethane membranes for on-demand intravaginal drug delivery**

Scheme 1. Synthesis scheme for pH-sensitive polyurethanes. (PEG-HEP-HDI-PG and PEG-HEP-MDI-PG).



R_1: contain polyether (using polyethylene glycol (PEG))
R_2: contain long alkyl chain (using hexamethylenediisocyanate (HDI) or hexamethylene di-p-phenyl diisocyanate (MDI))
R_3: contain tertiary amine group (using Bis-1,4-(hydroxyethyl)piperazine (HEP))
R_4: contain alkyl chain (using propylene glycol (PG))

- **Reversibly pH-responsive polyurethane membranes for on-demand intravaginal drug delivery**

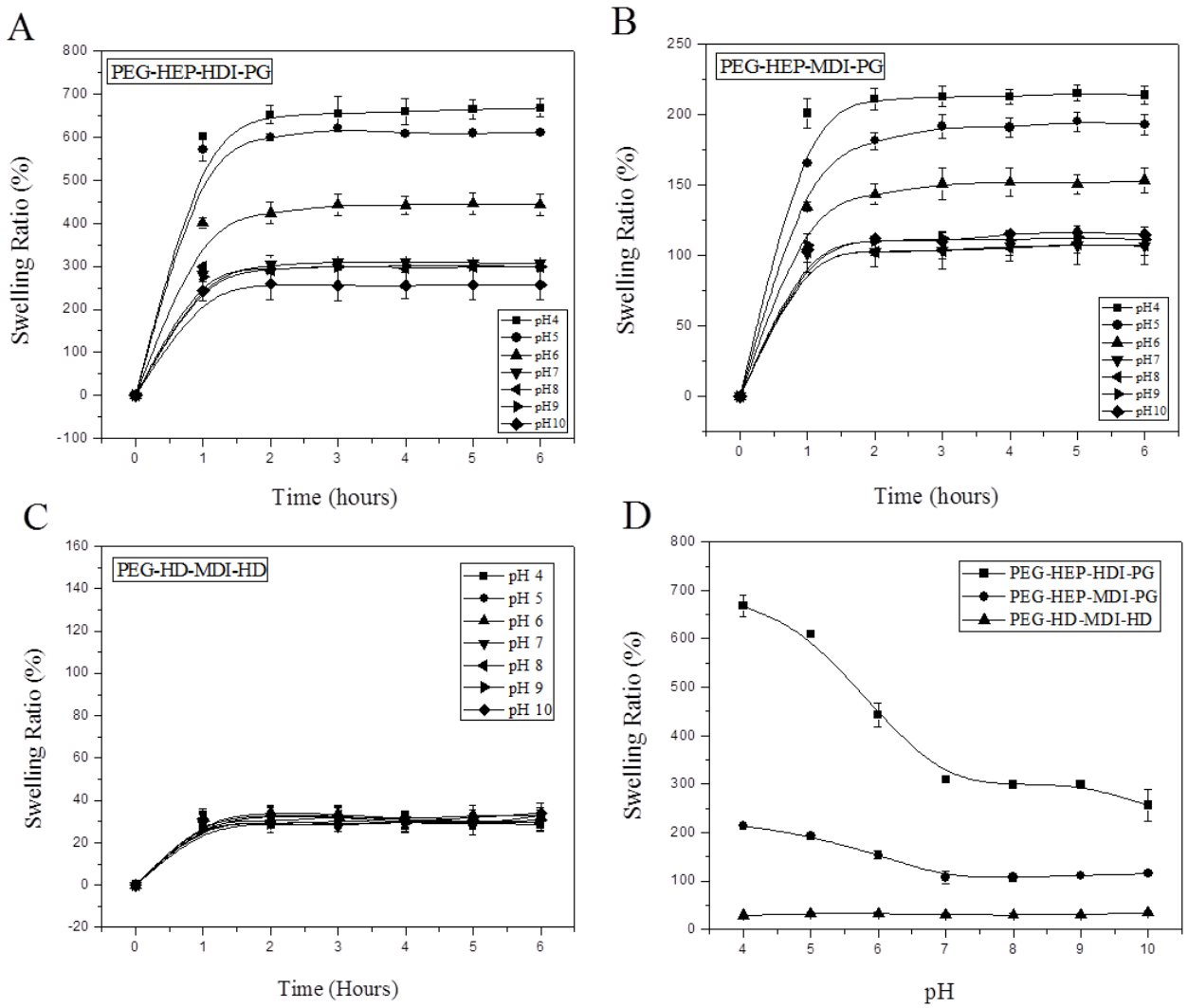
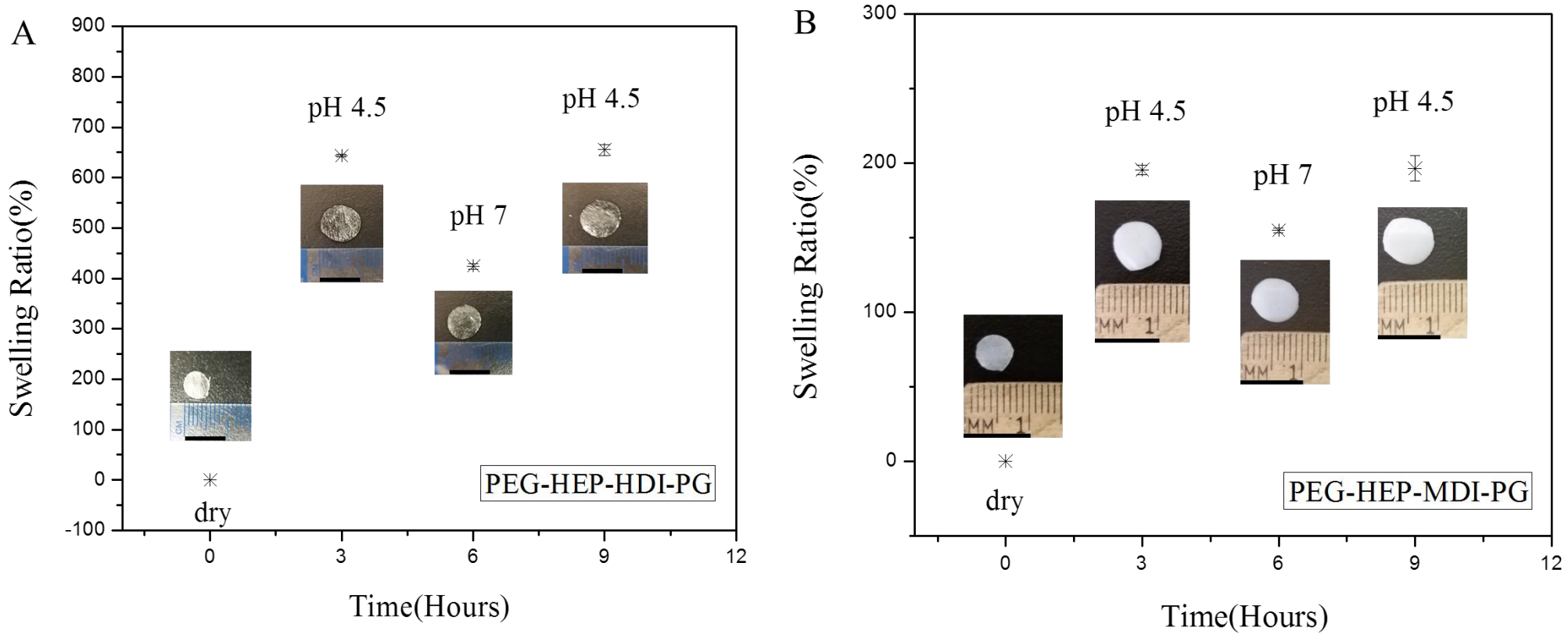


Figure 6. Swelling ratio of PU membranes in various pH buffers at 37 °C.
(A) PEG-HEP-HDI-PG membrane,
(B) PEG-HEP-MDI-PG membrane,
(C) non-pH-responsive PEG-HD- MDI-HD control,
and (D) Comparison of PEG-HEP-HDI-PG membrane, PEG-HEP-MDI-PG membrane, and non-pH-responsive PEG-HD-MDI-HD control (swelling ratio after immersed for 3 h at different pH).
Data is expressed as mean ± SD; n = 3.

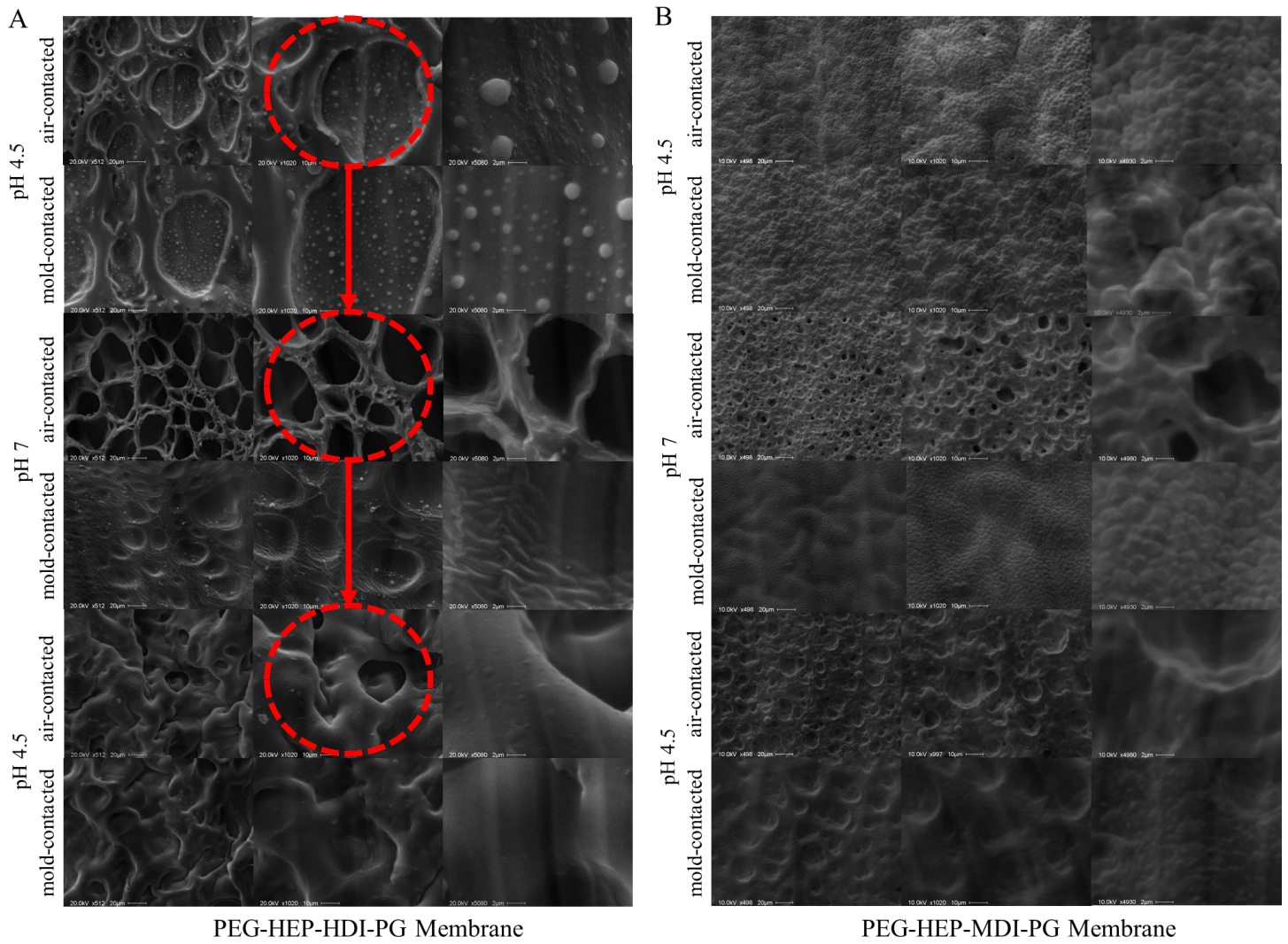
Acta Biomaterialia, 47: 100-112, 2017

- **Reversibly pH-responsive polyurethane membranes for on-demand intravaginal drug delivery**

Figure 7. Reversible swelling ratio change of pH-responsive PU membranes (A) PEG-HEP-HDI-PG membrane and (B) PEG-HEP-MDI-PG membrane at 37 °C. Data is expressed as mean ± SD; n = 3.



- **Reversibly pH-responsive polyurethane membranes for on-demand intravaginal drug delivery**



PEG-HEP-HDI-PG Membrane

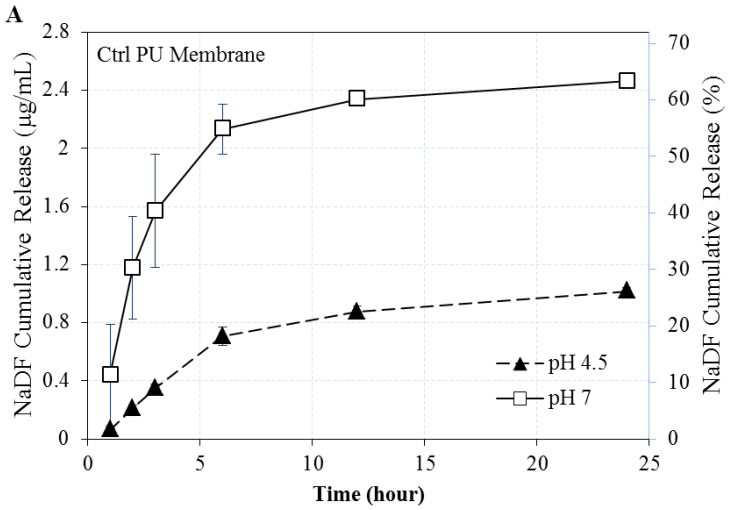
PEG-HEP-MDI-PG Membrane

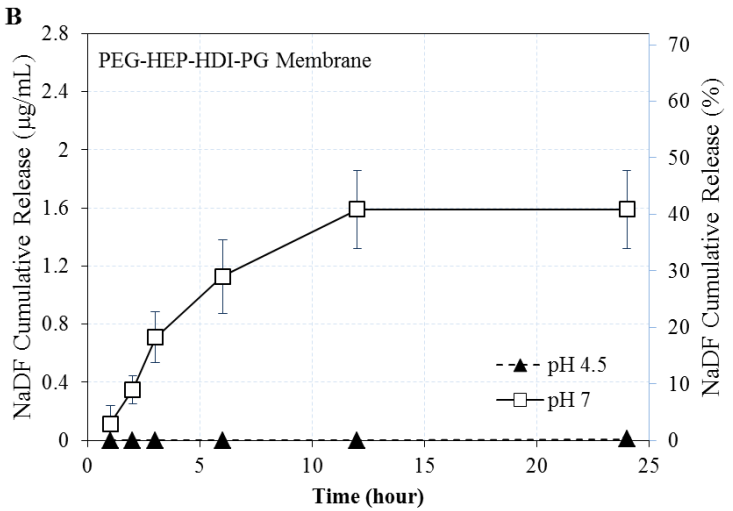
Figure 8. Reversible morphology change of pH-responsive PU membranes
(A) PEG-HEP-HDI-PG membrane and
(B) PEG-HEP-MDI-PG membrane.

Air-contacted surface during casting membrane and mold-contacted surface during casting membrane were characterized.

Acta Biomaterialia, 47: 100-112, 2017

- **Reversibly pH-responsive polyurethane membranes for on-demand intravaginal drug delivery**





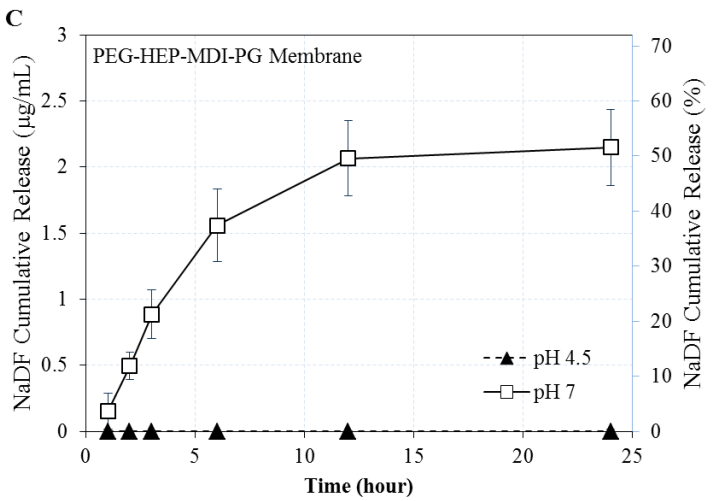


Figure 9. *In vitro* drug permeation study of NaDF using (A) non-pH-responsive PEG- HD-MDI-HD membrane as a control (Ctrl PU Membrane), (B) PEG-HEP-HDI-PG membrane, and (C) PEG-HEP-MDI-PG membrane. Cumulative release of NaDF both in concentration (lg/mL) and percentage (%) over 24 h was evaluated at both pH 4.5 and pH 7. Temperature was maintained at 37 °C. Data is expressed as mean ± SD; n = 3. Ctrl, control; PU, polyurethane.

Acta Biomaterialia, 47: 100-112, 2017

- **Reversibly pH-responsive polyurethane membranes for on-demand intravaginal drug delivery**

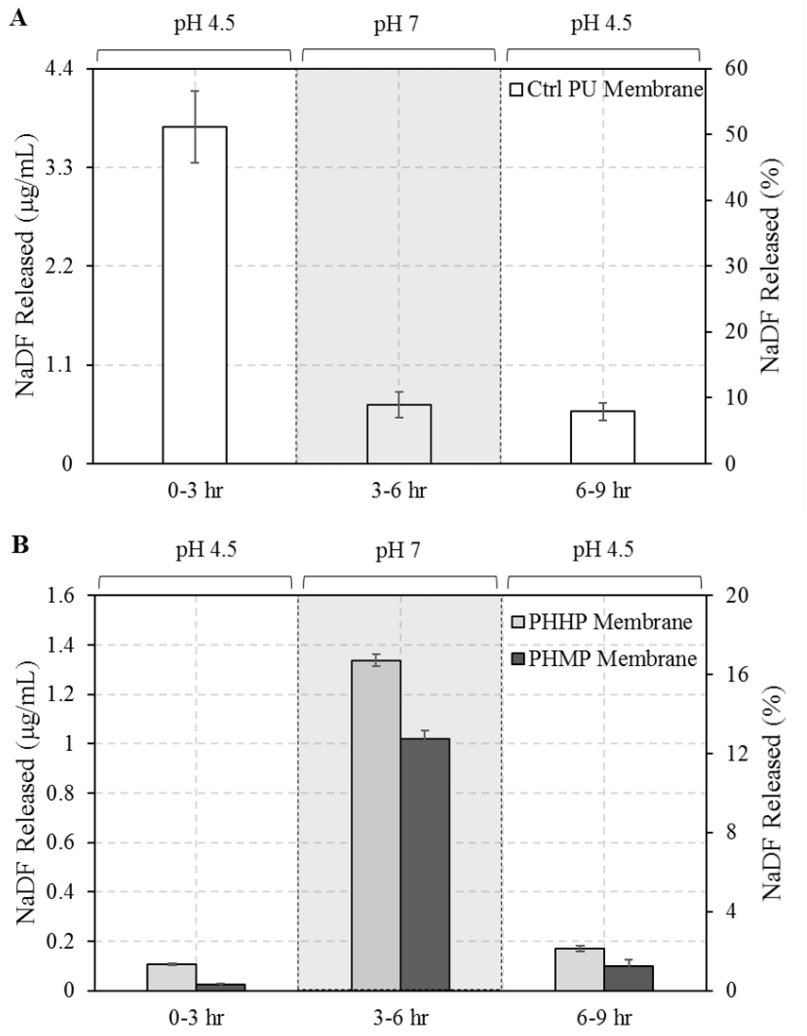


Figure 10. *In vitro* reversible drug permeation studies using (A) non-pH-responsive PEG-HD-MDI-HD membrane as a control (Ctrl PU Membrane) and
(B) pH-responsive PEG- HEP-HDI-PG membrane (PHHP Membrane) and PEG-HEP-MDI-PG membrane (PHMP Membrane).
Reversal permeation studies were conducted by shifting the pH of the acceptor chamber medium from 4.5 to 7 every 3 h.
Temperature was maintained at 37 °C. Data is expressed as mean ± SD; n = 3.

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- **Reversibly pH-responsive polyurethane membranes for on-demand intravaginal drug delivery**

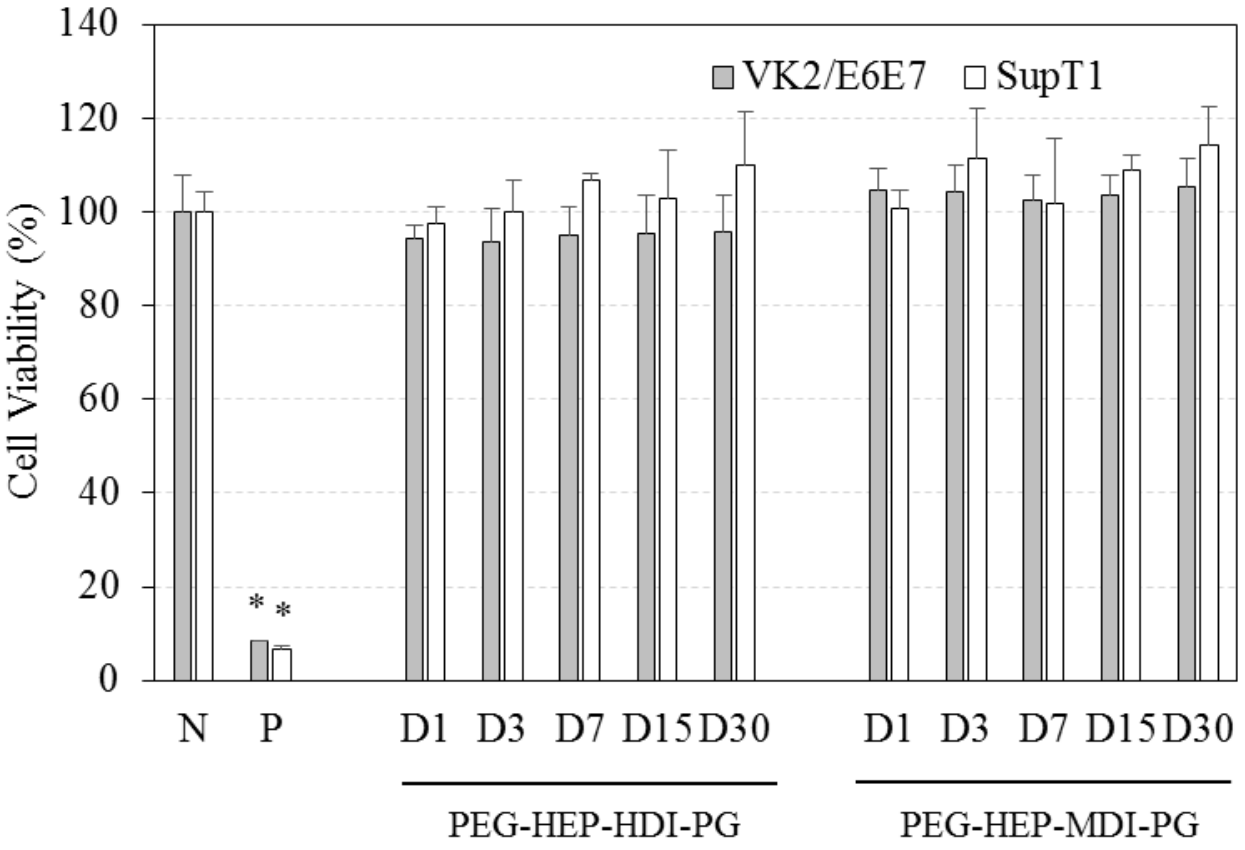


Figure 11. *In vitro* biocompatibility evaluations of the synthesized pH-responsive membranes using VK2/E6E7 and Sup T1 cells.
MTS assay was performed to determine the cell viability. MTS, a tetrazolium compound, can be bioreduced by live cells into a colored formazan product measurable at 490 nm.
Data is normalized to the negative control and expressed as mean ± SD; n = 4.
Cells cultured in plain medium were used as negative control.
1 M acrylamide dissolved in regular cell culture medium was used to induced cell death in positive control groups. N, negative control; P, positive control. $p < 0.05$ versus negative control.

Acta Biomaterialia, 47: 100-112, 2017

- **Reversibly pH-responsive polyurethane membranes for on-demand intravaginal drug delivery**

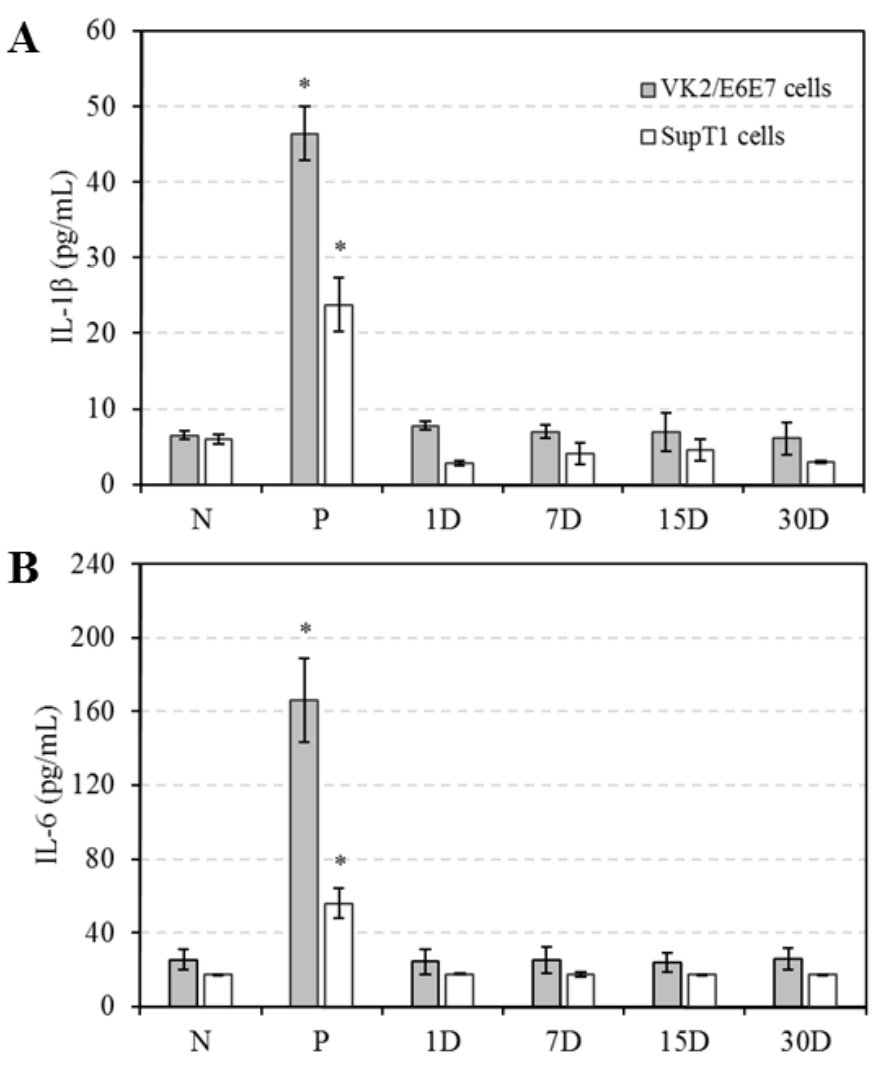


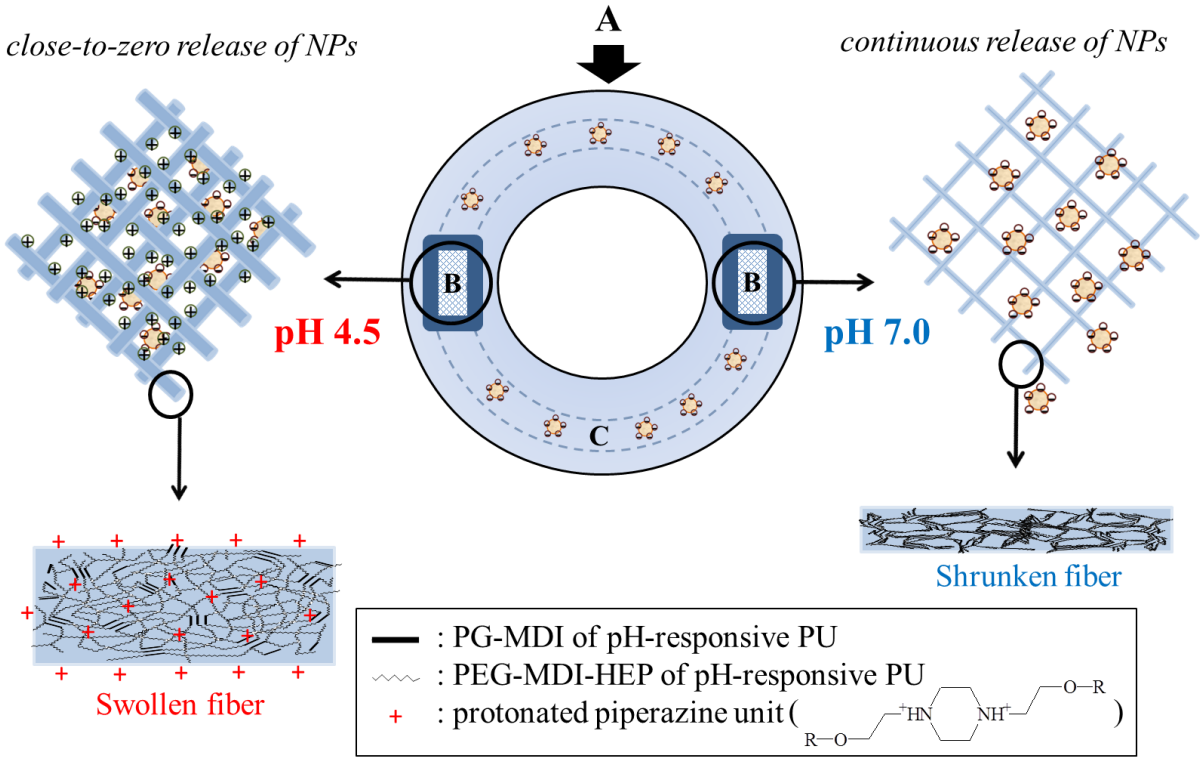
Figure 12. Impact of pH-responsive PU membranes on proinflammatory cytokine production (A) Interleukin (IL)-1b, (B) IL-6, and (C) IL-8 production.
Plain medium was used as a negative control, and 200 lg/mL of nonoxynol-9 or 50 lg/mL of lipopolysaccharide-treated cells were used as positive controls for IL-1b and IL-6/IL- 8, respectively.
Data is expressed as mean ± SD; n = 4. $p < 0.05$ versus negative control.

Acta Biomaterialia, 47: 100-112, 2017

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

; 본 연구에서는 합성된 신규 pH 민감성 폴리우레탄 (PEG-HEP-MDI-PG)을 IVR의 interconnected porous electrospun “window” membrane으로 제작하여, pH 변화에 따른 anti-HIV nanocarrier의 “on-demand” permeability를 확인 하였다.

Figure 13. 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.



Acta Biomaterialia, 82: 12-23, 2018

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

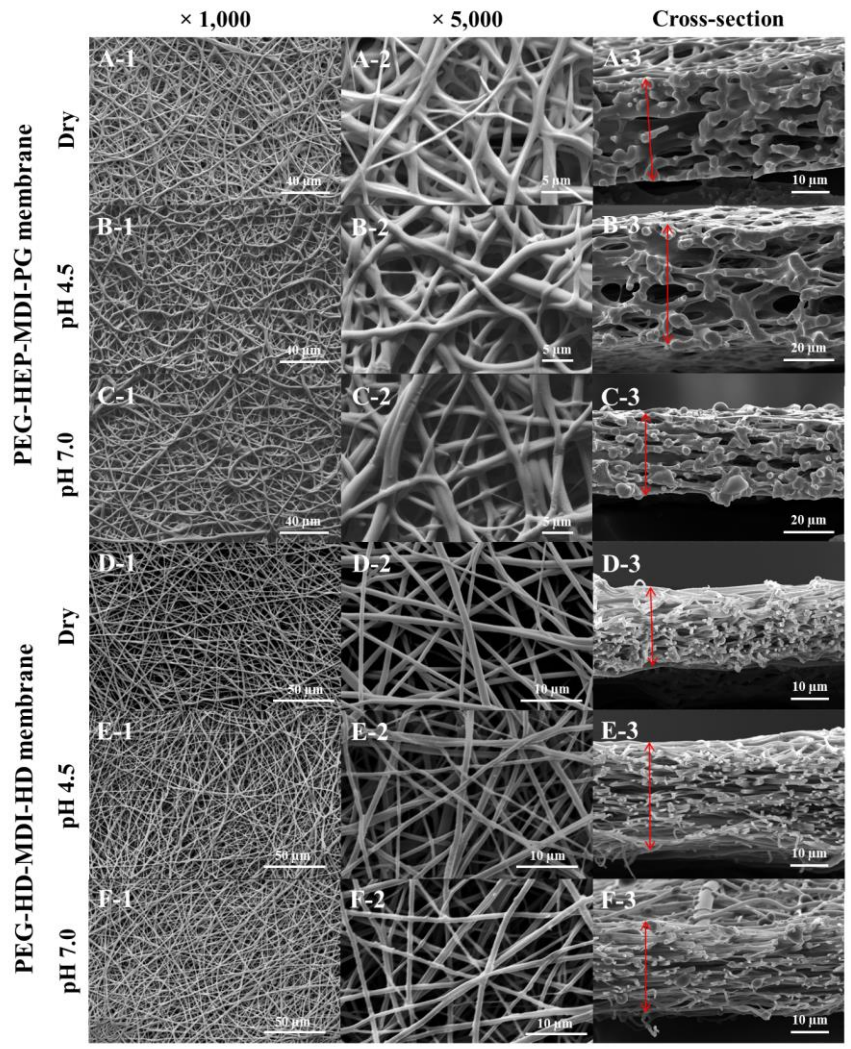


Figure 14. Morphology of electrospun porous pH-responsive PU (PEG-HEP-MDI-PG) membrane (DMF:THF = 3:7): dry (A), pH 4.5 (B), pH 7.0 (C), and control PU (PEG-HD-MDI- HD) membrane: dry (D), pH 4.5 (E), pH 7.0 (F). The red arrows represent the membrane thickness. Scale bars are shown in the images.

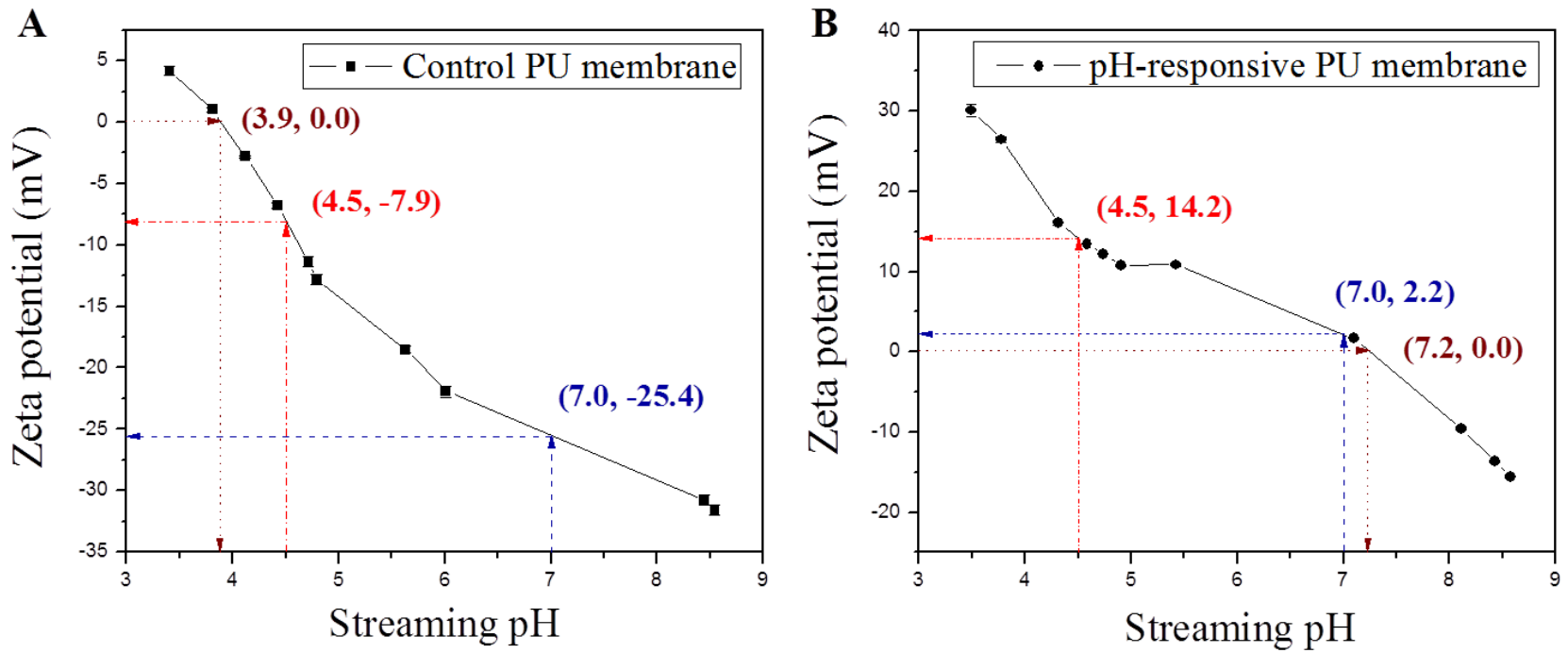
Table 1. Average pore size and diameter of electrospun porous pH-responsive (PEG-HEP-MDI-PG) and control (PEG-HD-MDI-HD) PU membrane at dry and wet conditions in VFS at pH 4.5 and 7.0.

		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

Acta Biomaterialia, 82: 12-23, 2018

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

Figure 15. 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.



Acta Biomaterialia, 82: 12-23, 2018

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

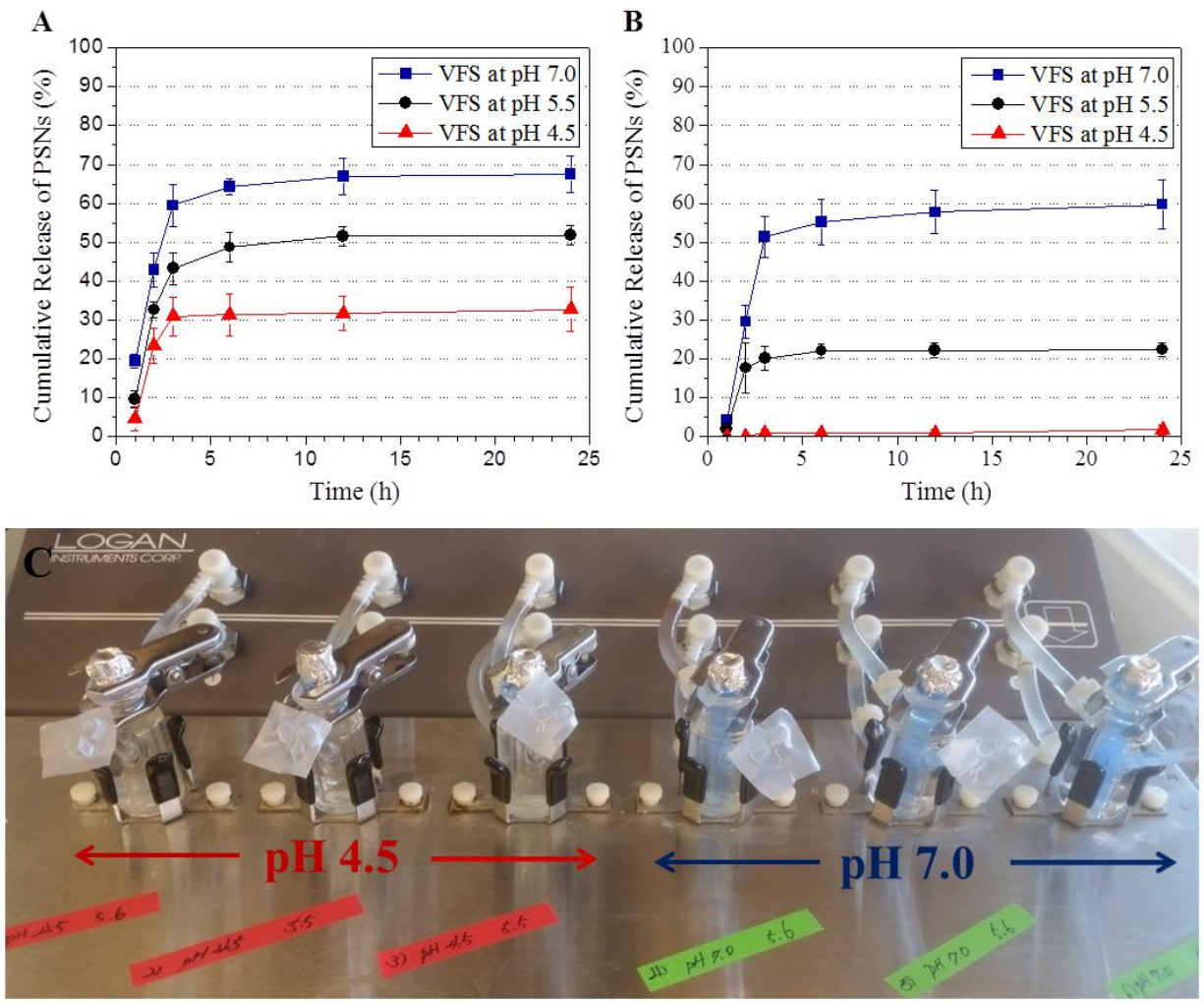


Figure 16. *In vitro* nanoparticle permeation studies of (A) control PU (PEG-HD-MDI-HD) membrane, (B) porous pH-responsive PU (PEG-HEP-MDI-PG) membrane, and (C) photo of the study using porous pH-responsive PU (PEG-HEP-MDI-PG) membrane by Franz cells.

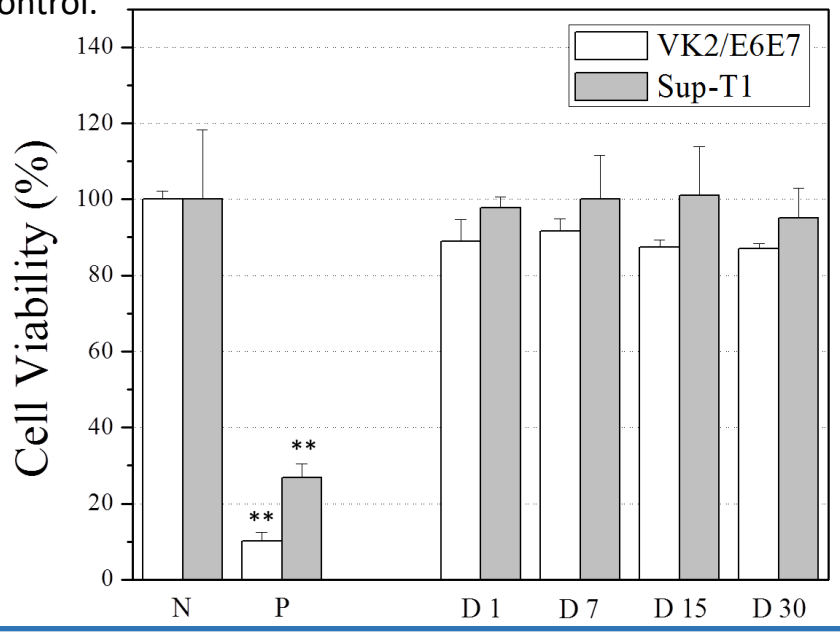
Cumulative release of the nanoparticle in percentage (%) for 24 h was evaluated at pH 4.5, pH 5.5, and pH 7.0.

Anionic blue-dyed nanoparticles (PSNs, 200 nm) were used.

Temperature was maintained at 37 °C.
Data are expressed as mean ± SD; n = 3. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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

Figure 17. *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.



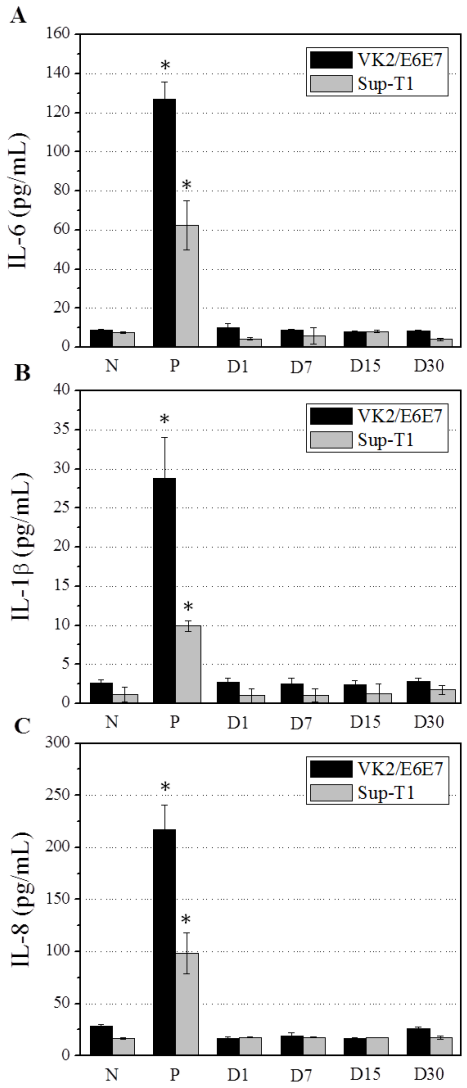


Figure 18. Impact of porous pH-responsive PU (PEG-HEP-MDI-PG) membrane on proinflammatory cytokine production (A) Interleukin IL-1b, (B) IL-6, and (C) IL-8 production.

Negative control was a plain medium, and positive control was 200 mg/mL of nonoxynol-9 or 50 mg/mL of lipopolysaccharide-treated cells for IL-1b and IL-6/IL-8, respectively.

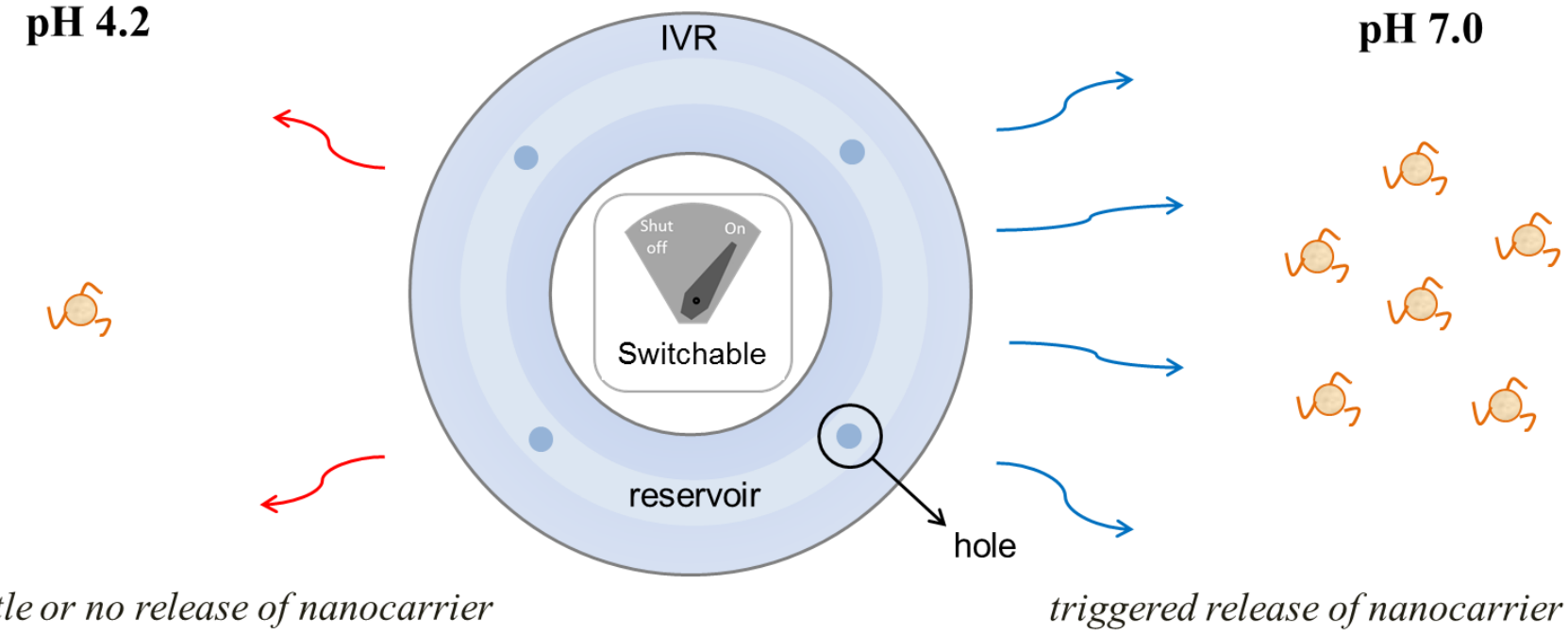
Data are expressed as mean ± SD; n = 3. One-way analysis of variance was performed for all results, with $p < 0.05$ considered as significant. N: negative control, P: positive control.

Acta Biomaterialia, 82: 12-23, 2018

- **Switchable On-Demand Release of a Nanocarrier from a Segmented Reservoir Type Intravaginal Ring Filled with a pH-Responsive Supramolecular Polyurethane Hydrogel**

; 본 연구에서는 신규 pH 민감성 폴리우레탄 (PEG-DMPA-HDI-PG) 하이드로젤을 제조하여 신규 합성된 intravaginal nanocarrier (PASP-PEG-Ph-Orange)와 IVR에 적용하여, pH-responsive switchable “on-demand” release를 확인하였다.

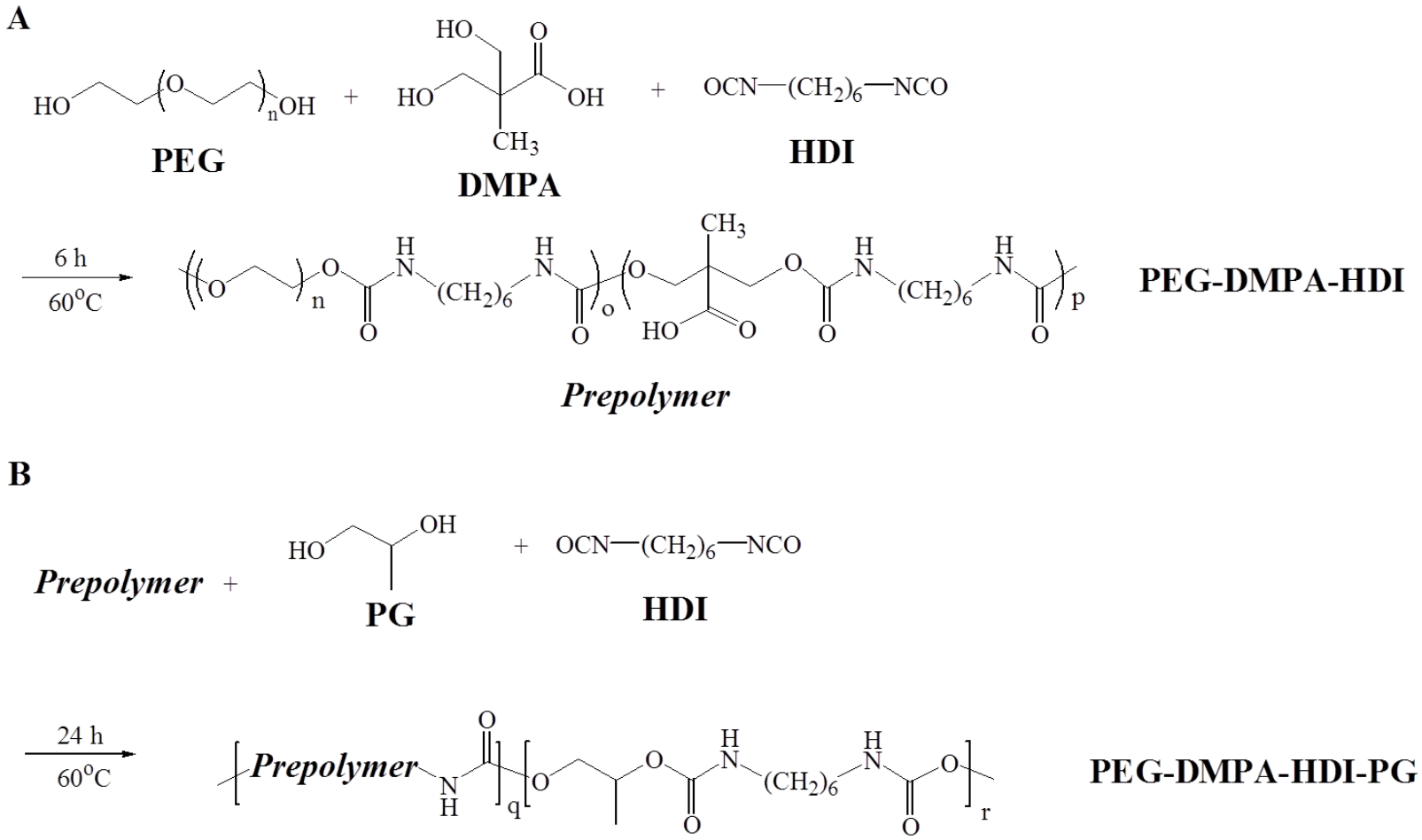
Figure 19. Diagram of the proposed switchable “on-demand” release of a nanocarrier from Intravaginal Ring Filled with a pH-Responsive Supramolecular Polyurethane Hydrogel.



ACS Applied Bio Materials, 1, 3: 652-662, 2018

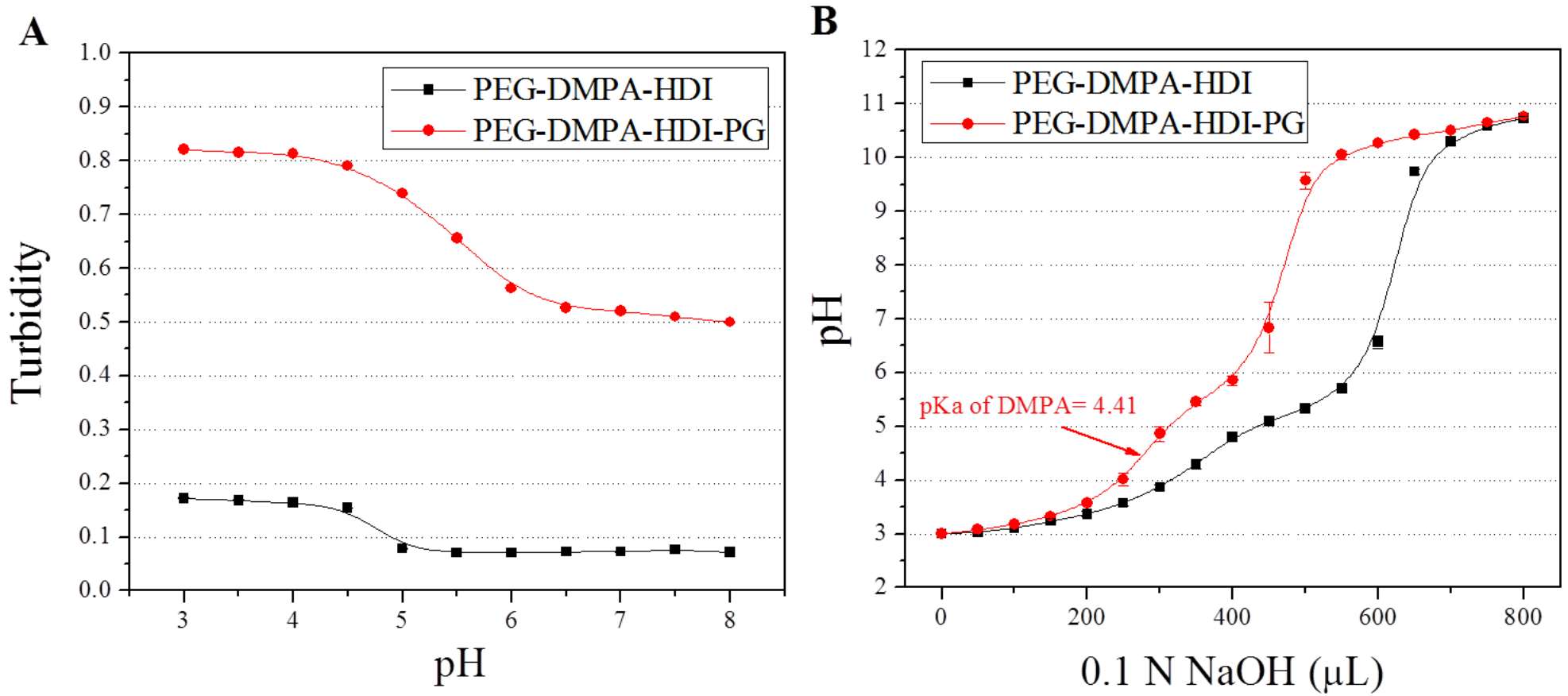
- **Switchable On-Demand Release of a Nanocarrier from a Segmented Reservoir Type Intravaginal Ring Filled with a pH-Responsive Supramolecular Polyurethane Hydrogel**

Scheme 2. Synthesis of pH-Sensitive PU Copolymers: (A) PEG-DMPA-HDI and (B) PEG-DMPA-HDI-PG.



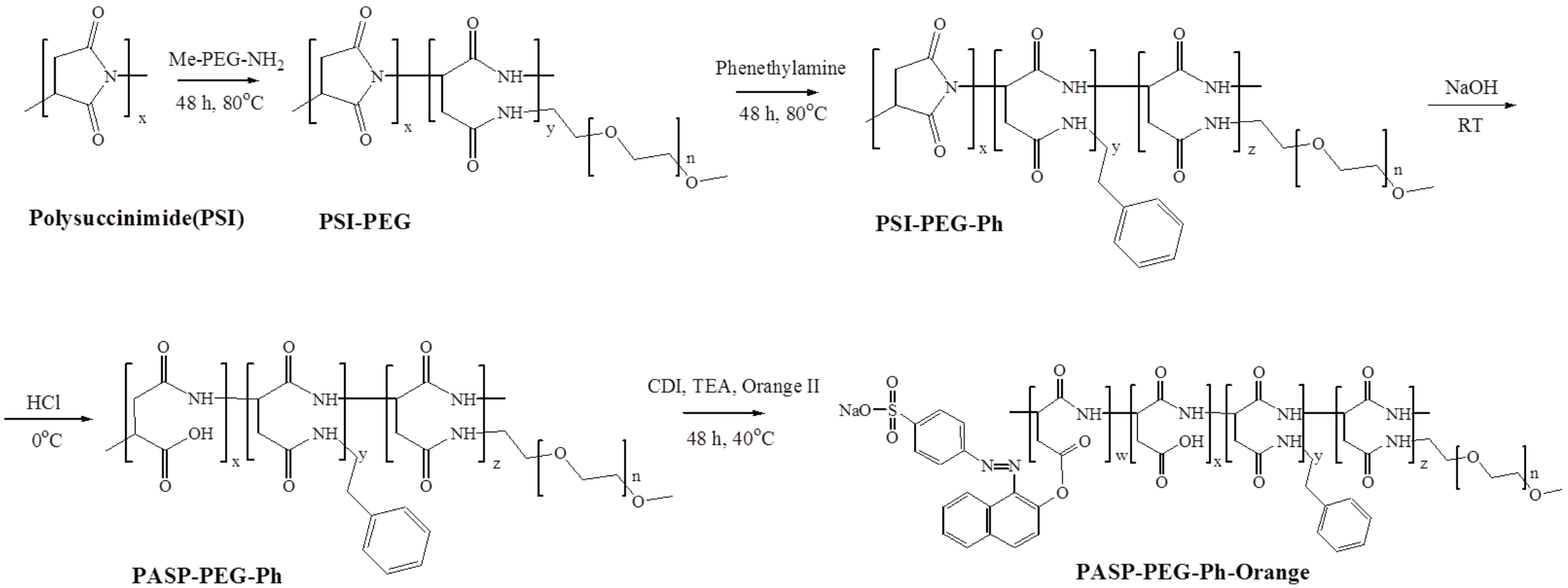
- **Switchable On-Demand Release of a Nanocarrier from a Segmented Reservoir Type Intravaginal Ring Filled with a pH-Responsive Supramolecular Polyurethane Hydrogel**

Figure 20. Turbidity change at 10 mg/mL (A) and acid−base titration profile at 1 mg/mL (B) of PEG-DMPA-HDI and PEG-DMPA-HDI-PG. Data is expressed as mean ± SD; n =3.



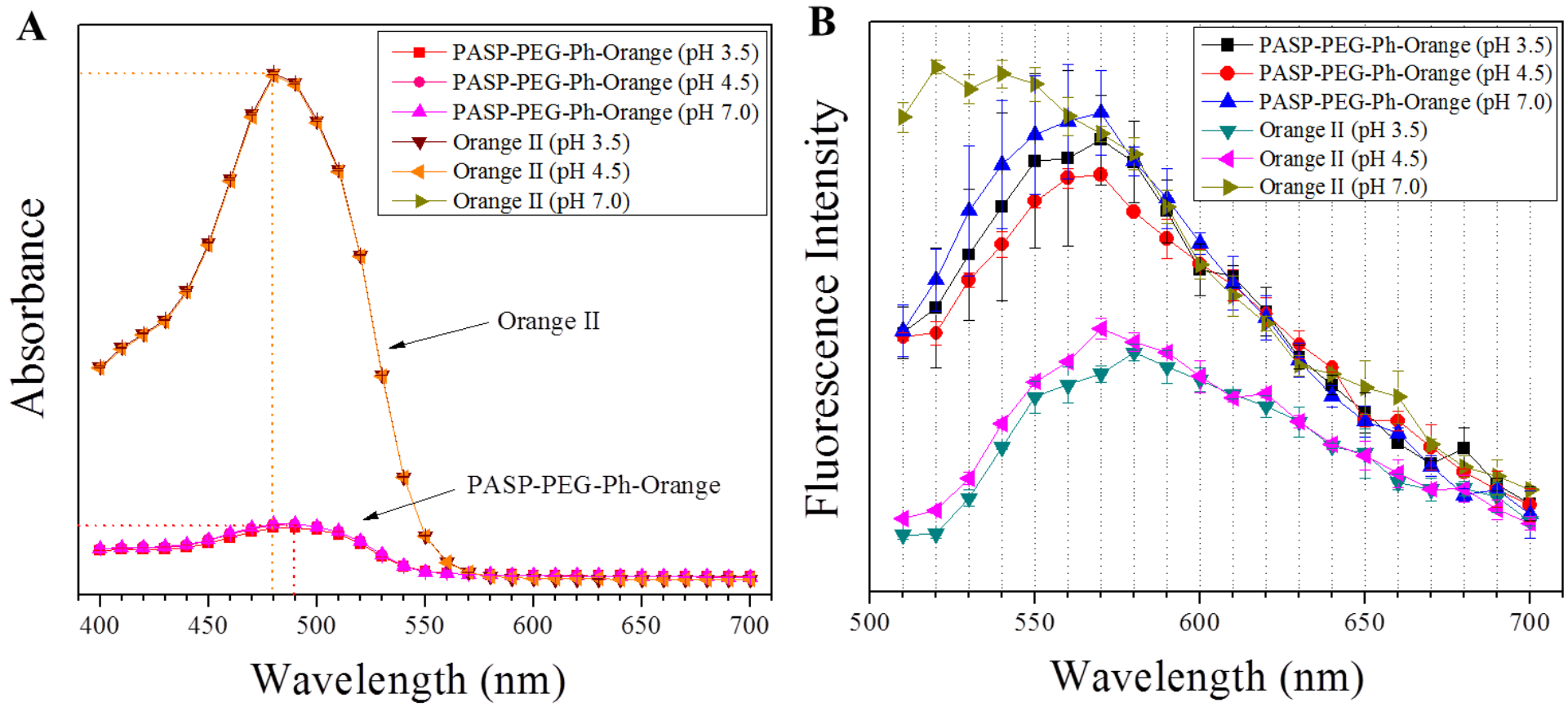
- **Switchable On-Demand Release of a Nanocarrier from a Segmented Reservoir Type Intravaginal Ring Filled with a pH-Responsive Supramolecular Polyurethane Hydrogel**

Scheme 3. Synthesis of Orange II Conjugated NP Based on PEGylated Polyaspartic Acid (PASP-PEG-Ph-Orange).



- **Switchable On-Demand Release of a Nanocarrier from a Segmented Reservoir Type Intravaginal Ring Filled with a pH-Responsive Supramolecular Polyurethane Hydrogel**

Figure 21. (A) UV−vis absorbance spectra of Orange II conjugated NPs (PASP-PEG-Ph-Orange) (0.1 mg/mL, in PBS, at pH 3.5, 4.5, 7.0, and 37 °C). (B) Fluorescence emission spectra of PASP-PEG-Ph-Orange with the excitation of 480 nm (0.1 mg/mL, in PBS, at pH 3.5, 4.5, 7.0, and 37 °C). Data is expressed as mean ± SD; n =3.



- **Switchable On-Demand Release of a Nanocarrier from a Segmented Reservoir Type Intravaginal Ring Filled with a pH-Responsive Supramolecular Polyurethane Hydrogel**

Figure 22. Diagram of the proposed use of pH-responsive supra- molecular PU hydrogels in reservoir-IVR for the switchable on- demand release of nanoparticles.

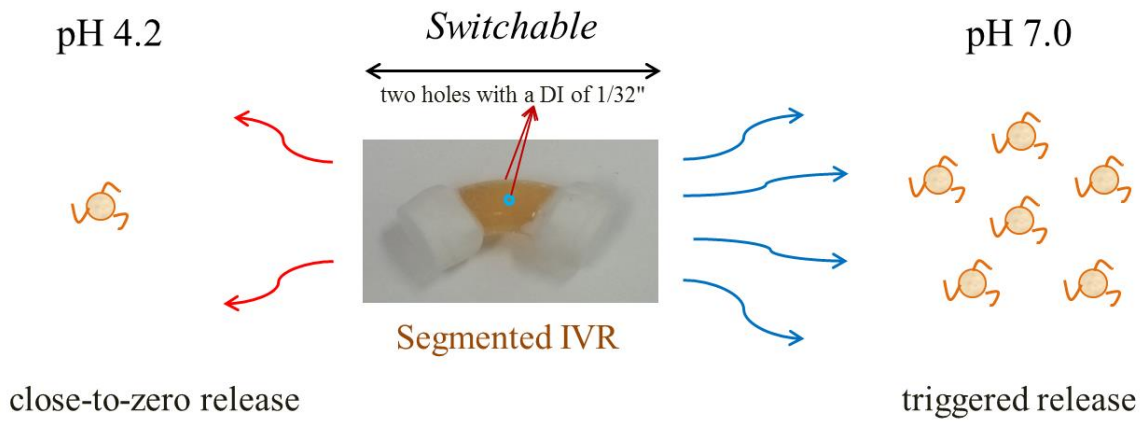


Figure 23. In vitro switchable on-demand release of PASP-PEG-Ph- Orange NPs using pH-responsive supramolecular PU hydrogels: (A) PEG-DMPA-HDI and (B) PEG-DMPA-HDI-PG. Data is expressed as mean ± SD; n =3.