화학공학소재연구정보센터

IP(Information Provider) 연구분야 보고서

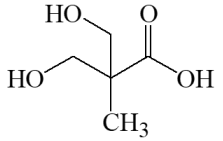
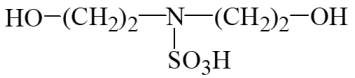
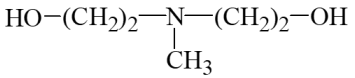
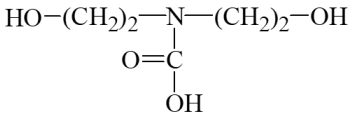
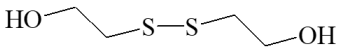
Bio 분야에서의 Polyurethane의 응용

4장. 자극감응성 폴리우레탄의 연구 동향

• pH-responsive polyurethanes (PUs)

: pH 반응성 (pH-responsive) 폴리 우레탄은 pH 민감성 폴리올 (polyol)이나 폴리아민(poly-amine)을 사용하여 합성한다. 이들 pH 민감성 polyol 또는 poly-amine은 폴리 우레탄의 합성 시 long-chain diol과 pre-polymer에 합성에 사용 되거나 chain extender로 사용 되어 질 수 있다. 합성 된 pH 반응성 폴리 우레탄의 주변 pH 변화에 대한 민감성은 폴리 우레탄의 소수성(hydrophobicity)/친수성(hydrophilicity) 비율과 polyol 또는 poly-amine의 고분자 사슬에서의 분포에 따라 조절 될 수 있다. 한 예로, 폴리우레탄의 소수성/친수성 비율 또는 수소 결합의 정도를 크게 하여, pH 반응성 shape-maintain/dissolved 특성을 low-swelling/high-swelling 특성으로 변화 시킬 수 있다. 대표적이 pH 민감성 polyol은 Table 1과 같다.

Table 1. pH-sensitive polyols for pH-Responsive polyurethane synthesis.

Chemical name	Structure	pKa
2,2-Bis(hydroxymethyl)propionic acid (DMPA)		4.41
bis-1,4-(hydroxyethyl)piperazine (HEP)		6.4
N,N-bis (2-hydorxyethyl)-2-amino- ethane-sulfonic acid (BES)		7.1
2,2- (methylimino) diethanol (MIDE)		8.52
Bisine		8.3
Bis(2-hydroxyethyl)- disulfide (2-HDS)		6.3

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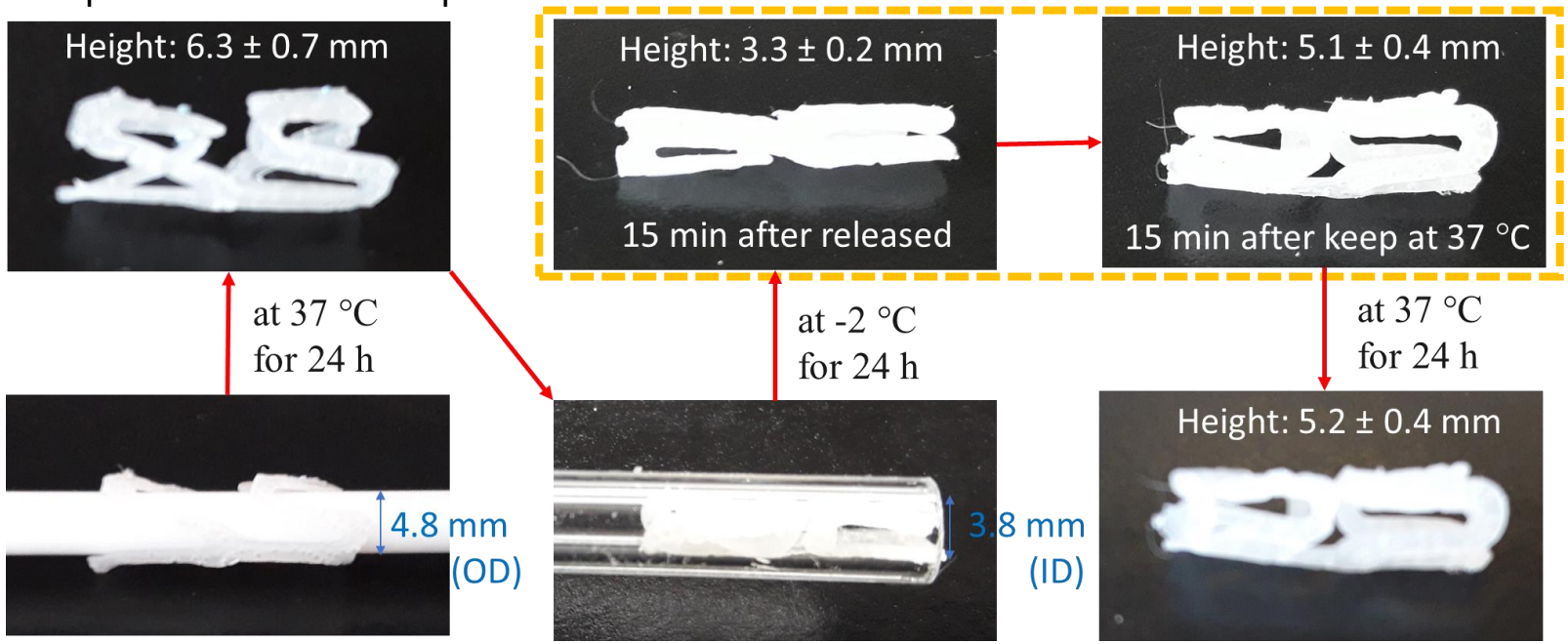
• Thermoresponsive polyurethanes (PUs)

: 그 화학적 구조에 따라 열가소성 (thermoplastic) 또는 열경화성 (thermoset) 폴리우레탄이 합성되어 진다. 열가소성 폴리우레탄은 열 분해에 대하여 비교적 안정하여, 열 사출 성형법 (hot melt extrusion) 에 의하여 다양한 형태로 성형 될 수 있다. 온도 반응성 (thermoresponsive) 폴리 우레탄은 온도에 민감한 soft segment와 안정한 hard segment 로 구성된 block copolymer로써의 특성에 기인한 특유의 물리화학적 특성을 지닌다. Soft segment는 switching temperature인 상전이 온도 (crystalline melting transition or glass transition temperature)을 지닌다.

Shape memory polyurethane은 온도 반응성 폴리 우레탄의 한 예로써, 특정 온도에서 기억되어진 형태를 회복하는 특성을 지닌다.

Figure 1. Simple test of thermoresponsive behavior.



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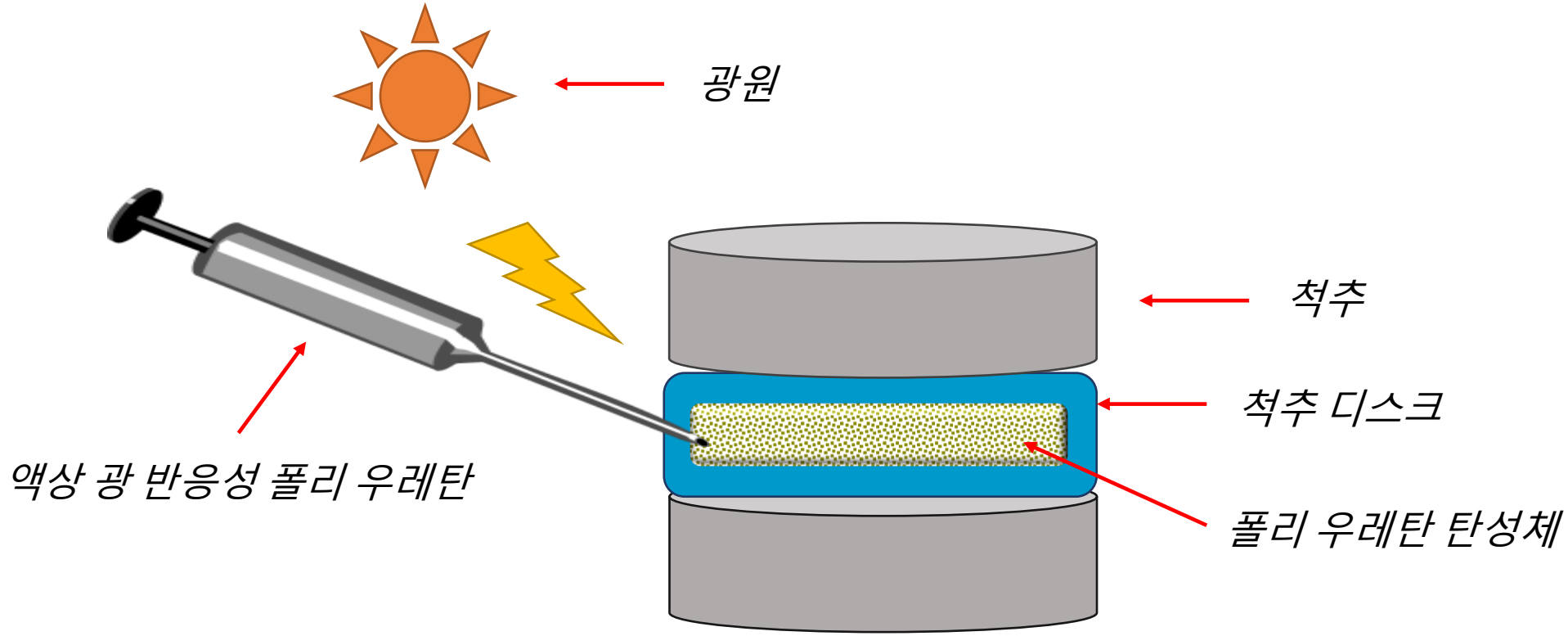
• Photoresponsive polyurethanes (PUs)

: 광 반응성 (photoresponsive) 폴리 우레탄은 광 민감성 (photosensitive) 반응기를 폴리 우레탄에 적용함으로써 합성 되어 진다. 이러한 photoresponsive 폴리 우레탄 합성 특성을 이용하여, photoresponsive injectable PU artificial nucleus pulposus도 개발 되어 질 수 있다.

Figure 2. Diagrams of photoresponsive injectable PU artificial nucleus pulposus.



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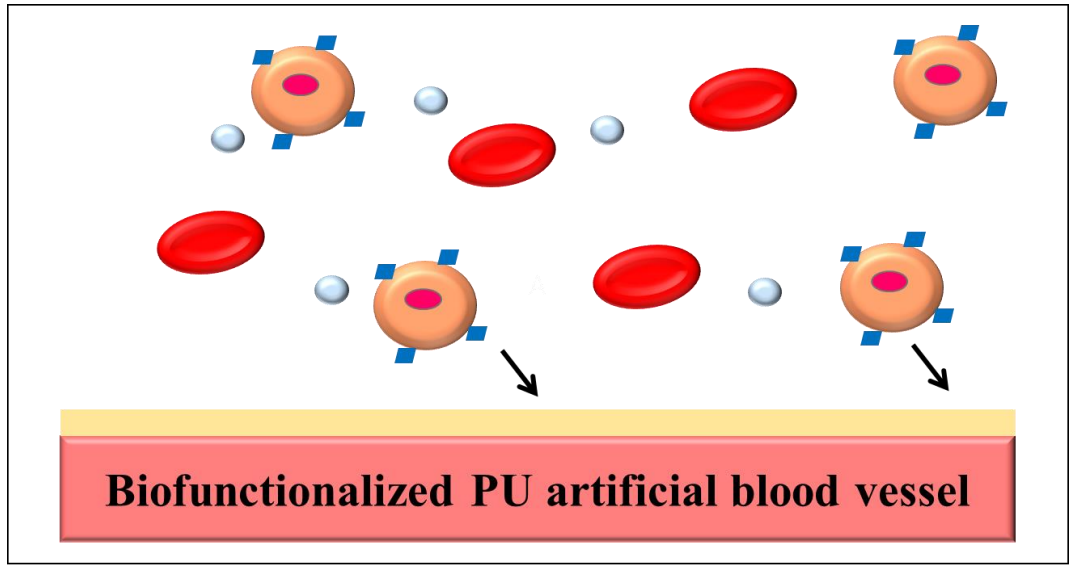
• Bioresponsive polyurethanes (PUs)

: Bioresponsive 폴리 우레탄은 생리적 신호나 병리학적 이상 특징 등 생리화학적 변화에 반응한다. 이러한 bioresponsive 폴리 우레탄은 효소 분해 부위 (enzyme-cleavable linkage), signal protein, 또는 vascular endothelial growth factor (VEGF)나 heparin과 같은 항응고제 (anticoagulant)를 도입함으로써 합성 할 수 있다.

이러한 bioresponsive 폴리우레탄 biomaterial의 한 예로, biofuntionalized된 lumen 표면을 지닌 폴리 우레탄 인공혈관은 혈류로부터 endothelial progenitor cell (EPC)의 표면 부착 및 활성화를 유도 함으로써, 내피화 (endothelialization)을 향상 시킬 수 있다.

Figure 3. Schematic representation of biofunctionalized PU artificial blood vessel enhancing EPC recruiting.



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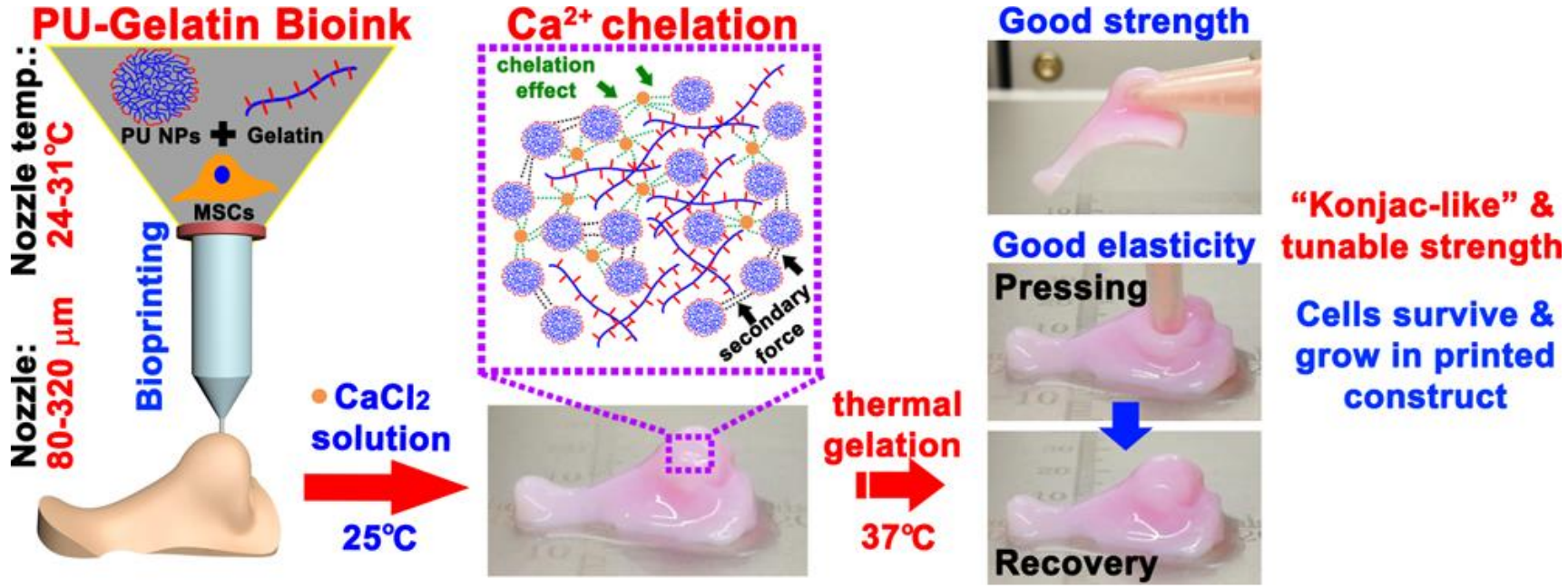
- **Double-Network Polyurethane-Gelatin Hydrogel with Tunable Modulus for High-Resolution 3D Bioprinting**

; 3D bioprinting은 바이오 잉크 (bioink)와 셀을 재생의학 (regenerative medicine) 또는 약물 screening에 응용되는 물질로 printing하는 기술을 말한다. 여기에 한 예로써, 생분해성 폴리우레탄-젤라틴 하이드로 젤 바이오 잉크에 관한 연구를 소개하고자 한다. 보고되어진 폴리 우레탄-젤라틴 하이드로 젤은 뛰어난 shear thinning과 빠른 strain recovery 특성에 기인하여, 24–31 °C에서 성공적으로 코 모양으로 printing 되었다. 또한 이 폴리 우레탄-젤라틴 하이드로 젤은 Mesenchymal stem cells (MSCs)과 print되어 good viability, high mobility, and ~200% proliferation ratio를 10일 동안 보여주었다.

Figure 4. Shear-thinning Polyurethane-Gelatin hydrogel for 3D biopringting.



** **shear thinning** is the non-Newtonian behavior of fluids whose viscosity decreases under **shear** strain*

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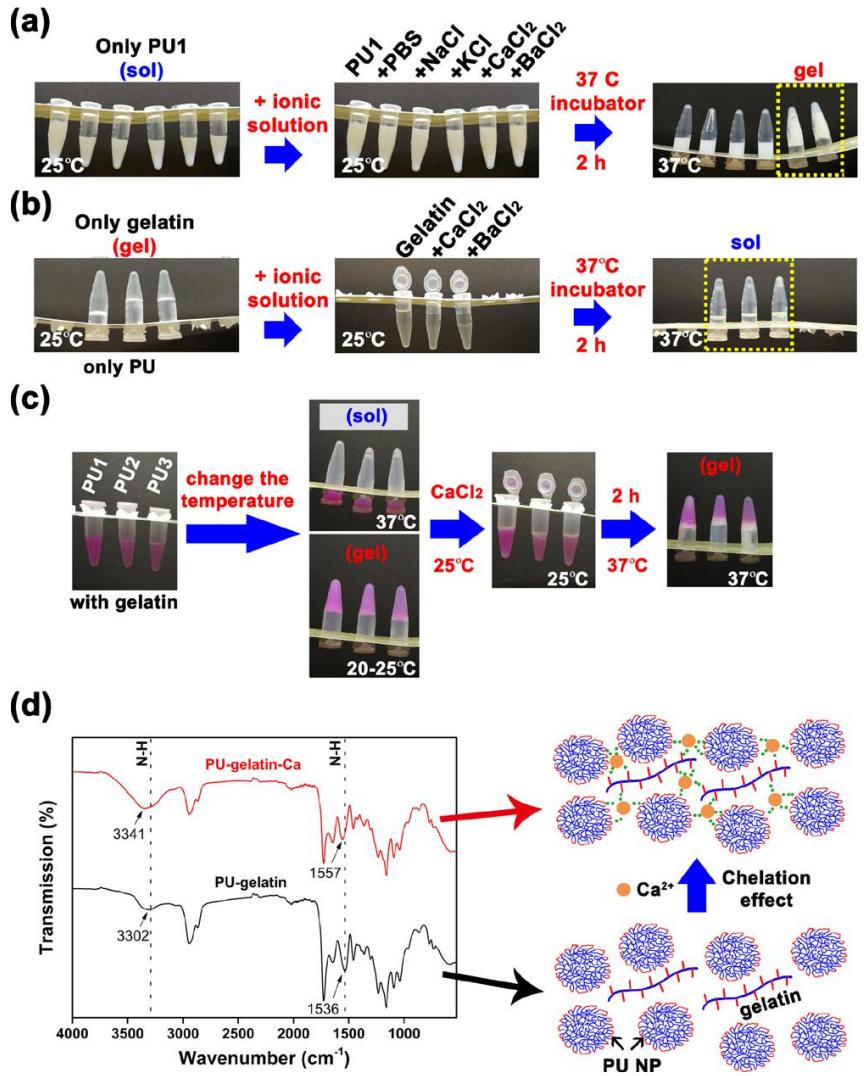

Figure 3. stabilization effect on PU and gelatin. (a) PU1 dispersion treated with various 0.2 N ionic solutions (PBS, NaCl, KCl, $CaCl_2$, and $BaCl_2$) showed different states, based on the gross appearance. Only that treated with divalent cations ($CaCl_2$ or $BaCl_2$) remained in the gel state at 37 °C. (b) The gelatin gel, even after the treatment of divalent cation solution ($CaCl_2$ or $BaCl_2$), returned to the sol state at 37 °C. (c) Gelation of various PU-gelatin hybrids (based on different PUs) after immersion in 0.2 N $CaCl_2$ solution. Prior to the treatment, all mixtures formed a gel at 25 °C, but the gel state did not endure at 37 °C (because of the gel-to-sol transition of gelatin). After the $CaCl_2$ treatment, all mixtures remained as hydrogels at 37 °C. (d) ATR-FTIR spectra of PU1-gelatin hydrogel before and after the treatment of $CaCl_2$. The abbreviation PU1-gelatin-Ca represents the PU-gelatin hydrogel after the treatment of $CaCl_2$.

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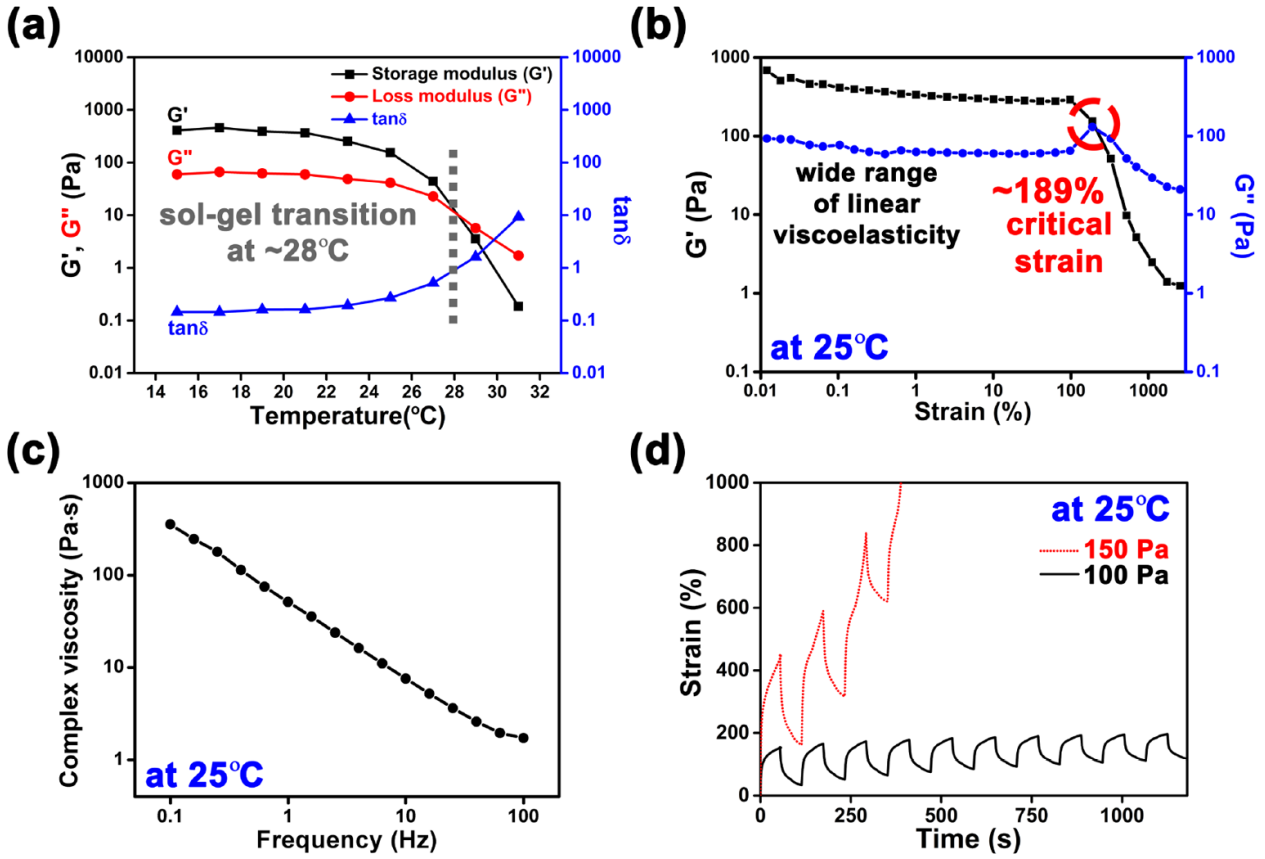
Figure 4. Rheological properties of PU1-gelatin bioink. (a) Gelation temperature measured in an oscillatory mode with 1% strain and 1 Hz frequency. (b) Dynamic strain sweep performed at 25 °C in oscillatory mode with 1 Hz frequency and 0.01−3000% strain. (c) Complex viscosity−frequency relationship curve measured at 25 °C in oscillatory mode with 1% strain and 0.1−100 Hz frequency. (d) Creep and creep recovery cycles (60 s each) at 25 °C showing a yield shear stress of ∼100 Pa. The ink was PU1-gleatin (4:1) with a solid content 12.5 wt %.



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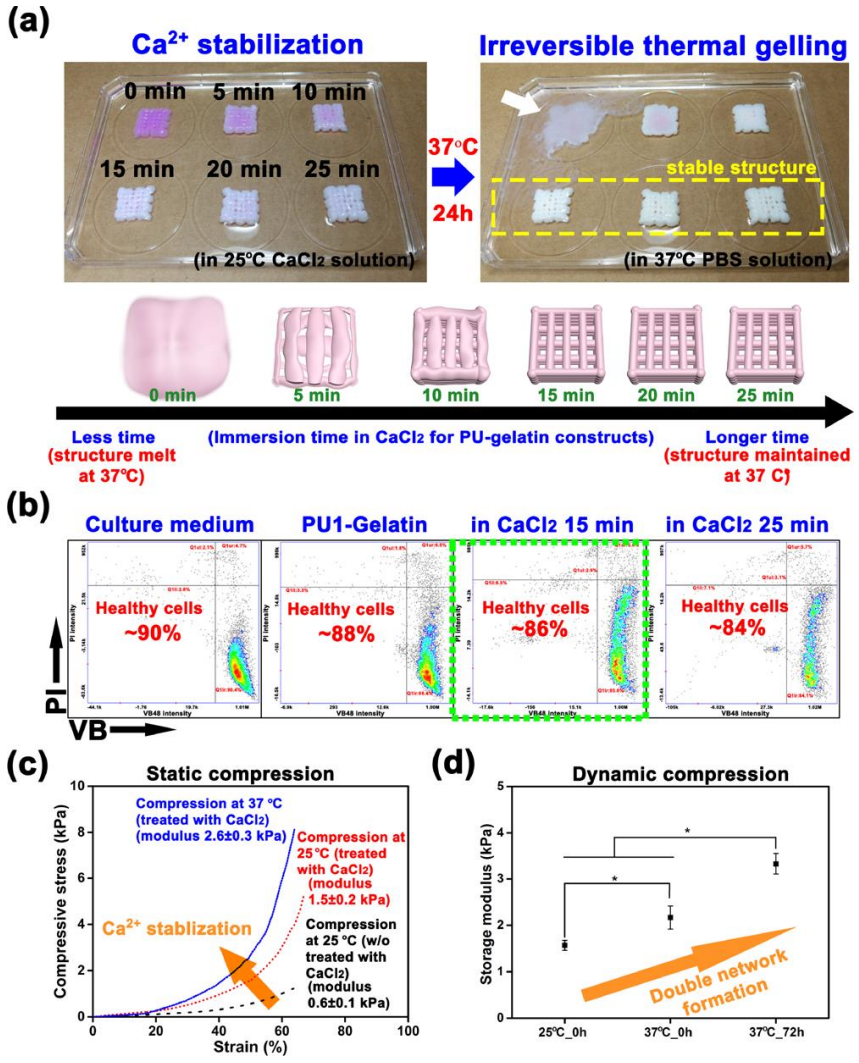

Figure 5. Structural stability of the cell-laden PU-gelatin constructs treated with the $CaCl_2$ solution for different times and the cell viability in the constructs.

(a) Gelation and structural stability of the printed constructs after moving to 37 °C. As shown by the white arrow, pristine PU-gelatin constructs did not endure 37 °C. A brief pretreatment (≥15 min) in $CaCl_2$ helped stabilize the structure at 37 °C. The printed hydrogel was PU1-gleatin (4:1) with a solid content 12.5 wt %.

(b) Health condition of human MSCs in PU1-gelatin hydrogels (with pretreatment of $CaCl_2$ for different times) at 37 °C. The number of live/dead cells was determined by image cytometry after VitaBright-48 (VB-48), acridine orange (AO), and propidium iodide (PI) tristaining. The VB-48 intensity indicates the glutathione level, which decreases in stressed cells and is an early hallmark of cell apoptosis.

(c) Static compression showing Ca^{2+} stabilization. Static compressive properties of PU1-gelatin treated with 0.2 N $CaCl_2$ solution for 15 min (25 °C) and measured by DMA at 25 or 37 °C.

(d) Dynamic compression showing physical network formation. The dynamic compressive properties of PU1-gelatin hydrogels treated with 0.2 N $CaCl_2$ solution for 15 min (25 °C) and further incubated in 37 °C PBS for 72 h measured by DMA at 25 or 37 °C (*$p < 0.05$).

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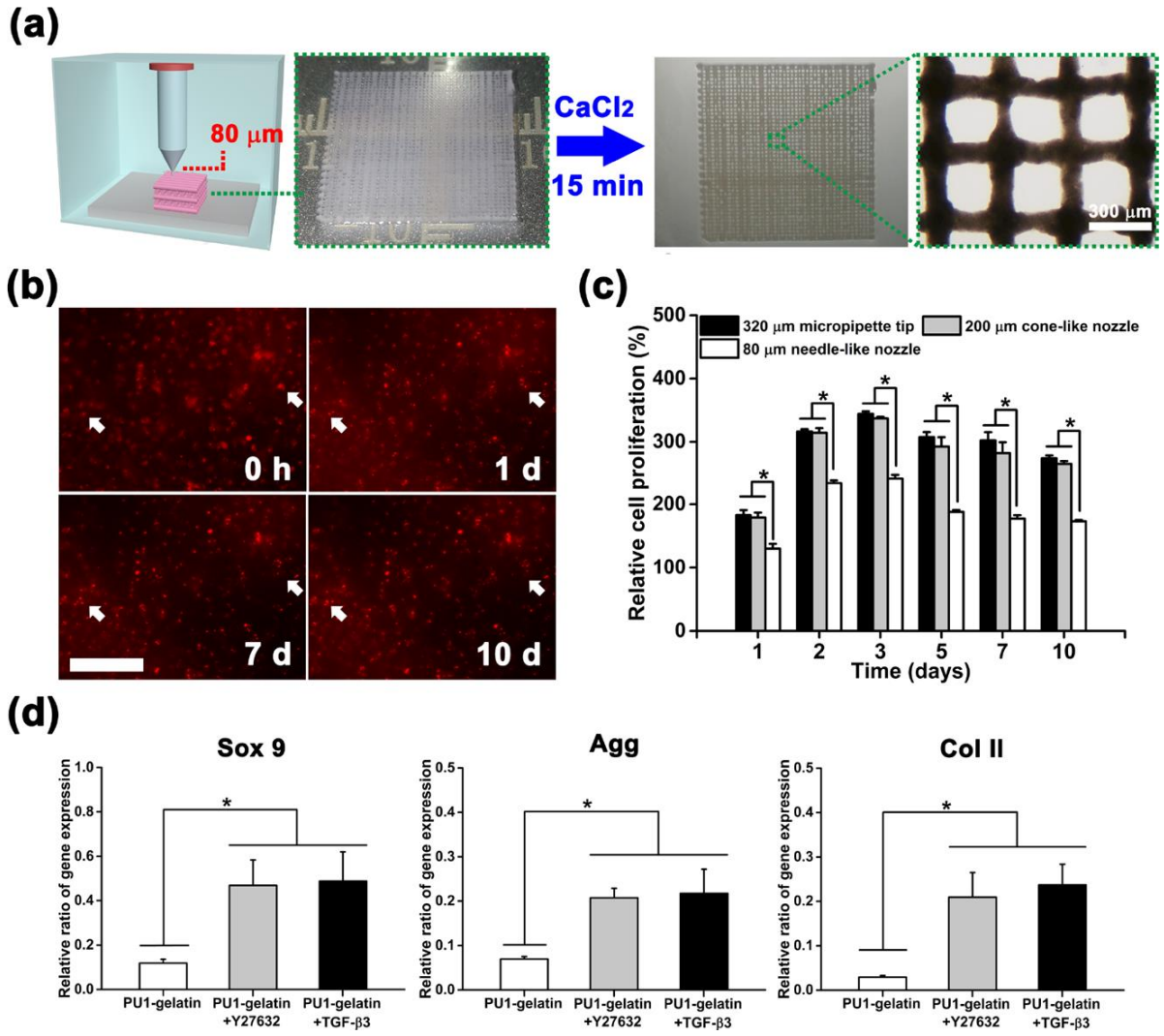

Figure 6. PU-gelatin bioink printability and the bioactivity of MSCs in the PU-gelatin constructs treated with $CaCl_2$ solution.
(a) High-resolution PU1-gelatin construct printed with an 80 μm diameter needle-like nozzle.
(b) Real-time observation for MSCs cultured in the PU1-gelatin construct for 10 days (printed with 200 μm nozzle) by a PKH26 fluorescence tracking dye. White arrows indicate the actively migrating cells. The scale bar is 100 μm.
(c) Proliferation of MSCs cultured in PU-gelatin constructs during the 10 day culture period by the WST-8 analysis. All data were normalized to those collected at the beginning of the 10 day culture (i.e., 0 day) for each group.
(d) Chondrogenic gene expression of MSCs printed with PU-gelatin and cultured for 10 days, with Y27632 or TGF-β3 as the chondrogenic induction medium.

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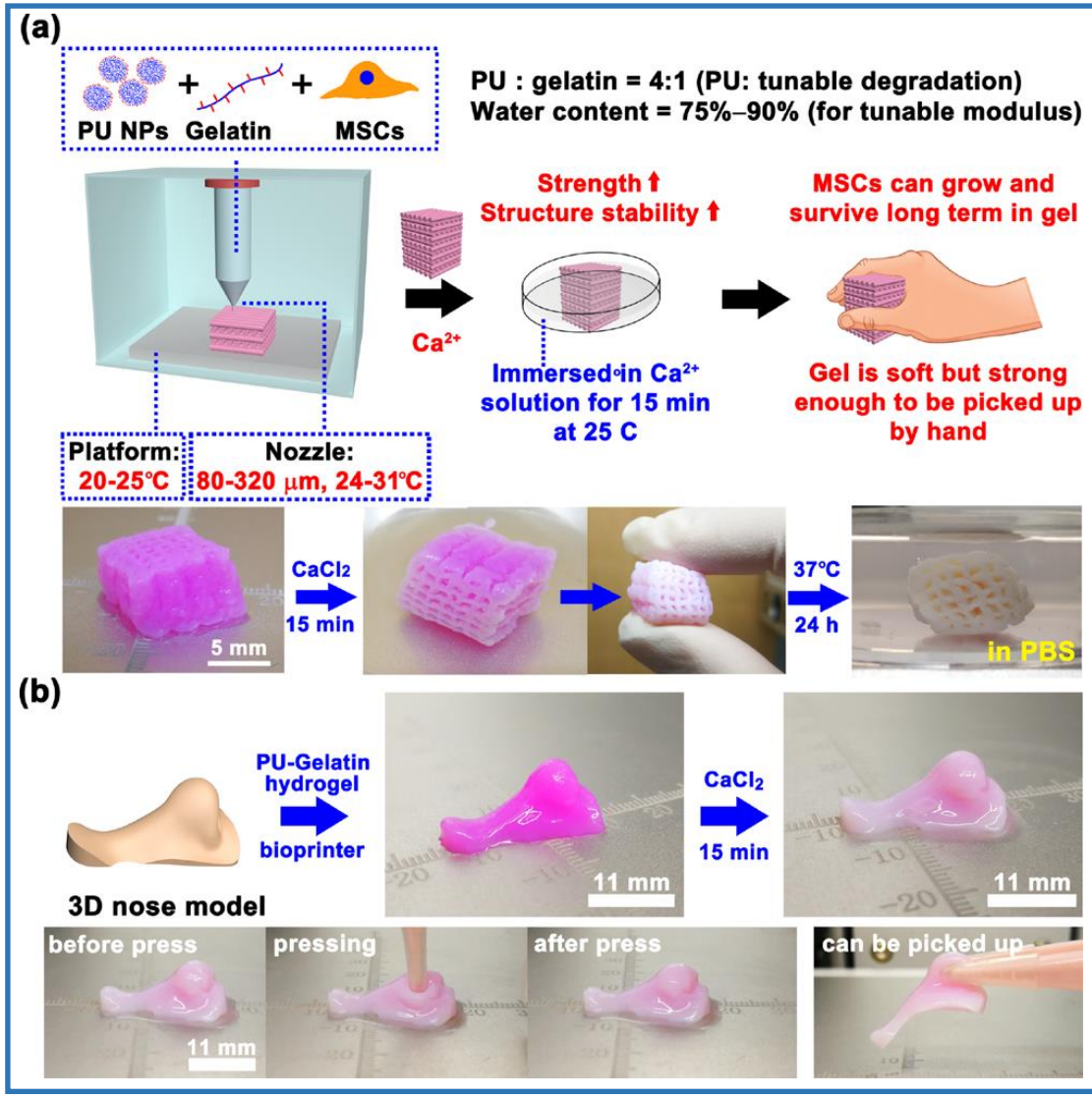

Figure 7. Characteristics of PU-gelatin bioink.

(a) Design and printing of the PU-gelatin bioink. The PU1-gelatin construct shown here was printed using a 200 μm diameter cone-like nozzle (50 layers).

(b) Nose-shaped PU1-gelatin construct printed using a 200 μm diameter needle-like nozzle (80 layers). The nose after pressing returned to the original status.

All of these constructs showed good elastic recovery and can be picked up by tweezers or hand.

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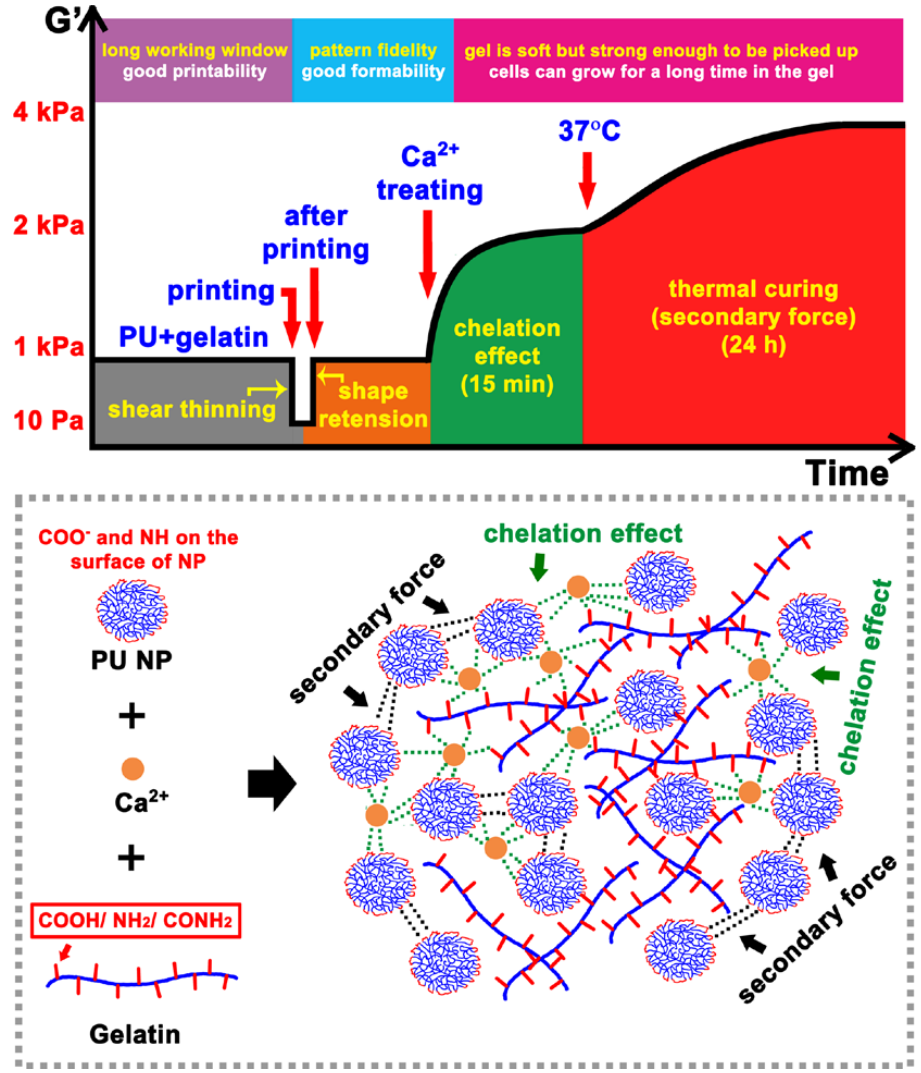

Figure 8. Possible two-stage gelation mechanism for the PU-gelatin double network. PU NPs in aqueous dispersion may interact with divalent cations to form a solid-like 3D network. The PU-gelatin mixture relies on the viscoelasticity of PU (and gelatin) for printing and the upper critical solution temperature (UCST) behavior of gelatin for stabilizing the printed constructs at room temperature. After the divalent cation treatment, the Ca^{2+} chelation forms between PU and gelatin to fix the PU-gelatin hydrogel. Therefore, when the PU-gelatin printed constructs are moved to a 37 °C incubator, the integrity and mechanical properties of the printed structure are either enhanced or maintained, based on the thermosensitivity of PUs (i.e., secondary force among PU NPs).

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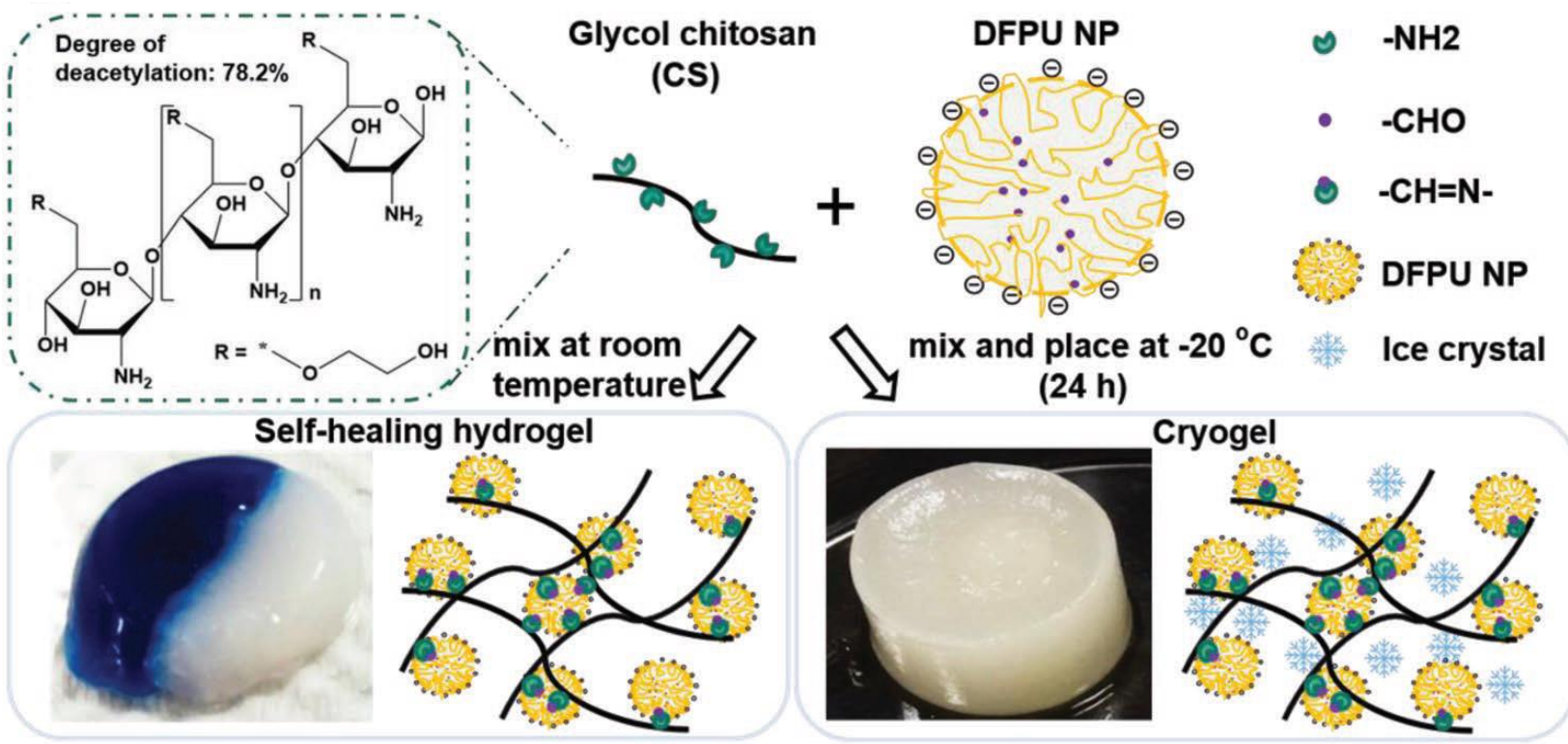
- **Self-Healing Hydrogels and Cryogels from Biodegradable Polyurethane Nanoparticle Crosslinked Chitosan**

; 하이드로젤은 특유의 수 친화적(high water retention)이며 soft한 특성에 기인하여 조직공학 (tissue engineering)에 광범위하게 사용된다. 아래의 연구 예에서는, 생분해성이며 다 기능성을 지닌 폴리 우레탄 (biodegradable and difunctional polyurethane, DFPU)을 합성하고, 이를 이용하여 injectable cryogel 또는 키토산 (chitosan, CS)과 가교된 self-healing 하이드로 젤을 만들고 이들의 특성을 확인하여 보았다.

Figure 9. Schematic representation of the simple process to form self-healing hydrogel or cryogel.



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- **Self-Healing Hydrogels and Cryogels from Biodegradable Polyurethane Nanoparticle Crosslinked Chitosan**

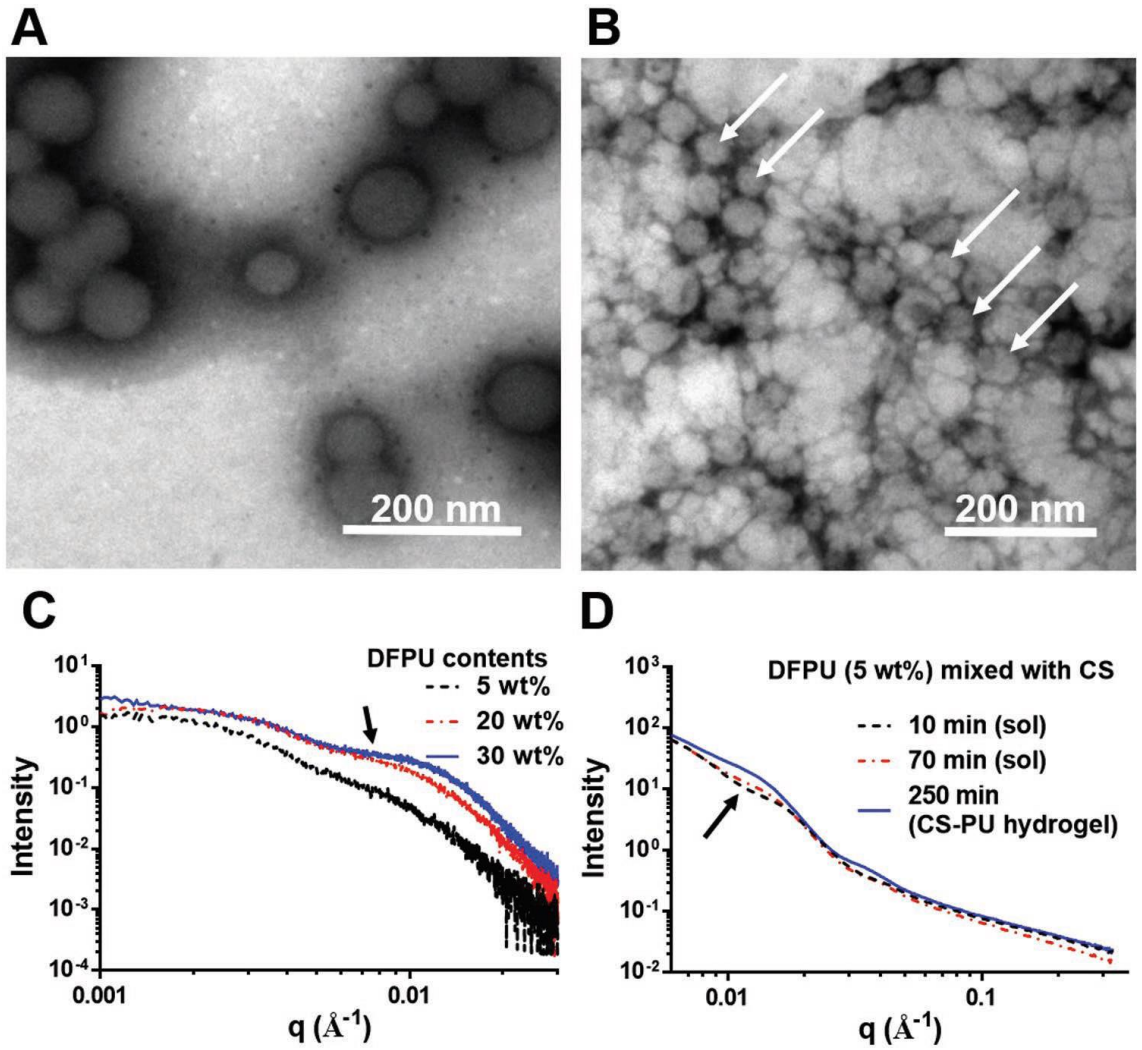

Figure 10. Characterization of DFPU by TEM and SAXS.
A)TEM images for DFPU NPs alone. The size was in the range of 30–50 nm.
B) TEM images for DFPU NPs mixed with glycol chitosan. The shape of DFPU nanoparticles changes when mixing with CS.
C) SAXS profiles (measured at 37 °C) for DFPU dispersions of various solid contents (5–30 wt%).
D) SAXS profiles for DFPU (5 wt%) mixed with twice the volume of glycol chitosan (3 wt%). The composition of the resulted CS-PU hydrogel is DFPU 1.7 wt%, glycol chitosan 2 wt%, and 96.3 wt% water.

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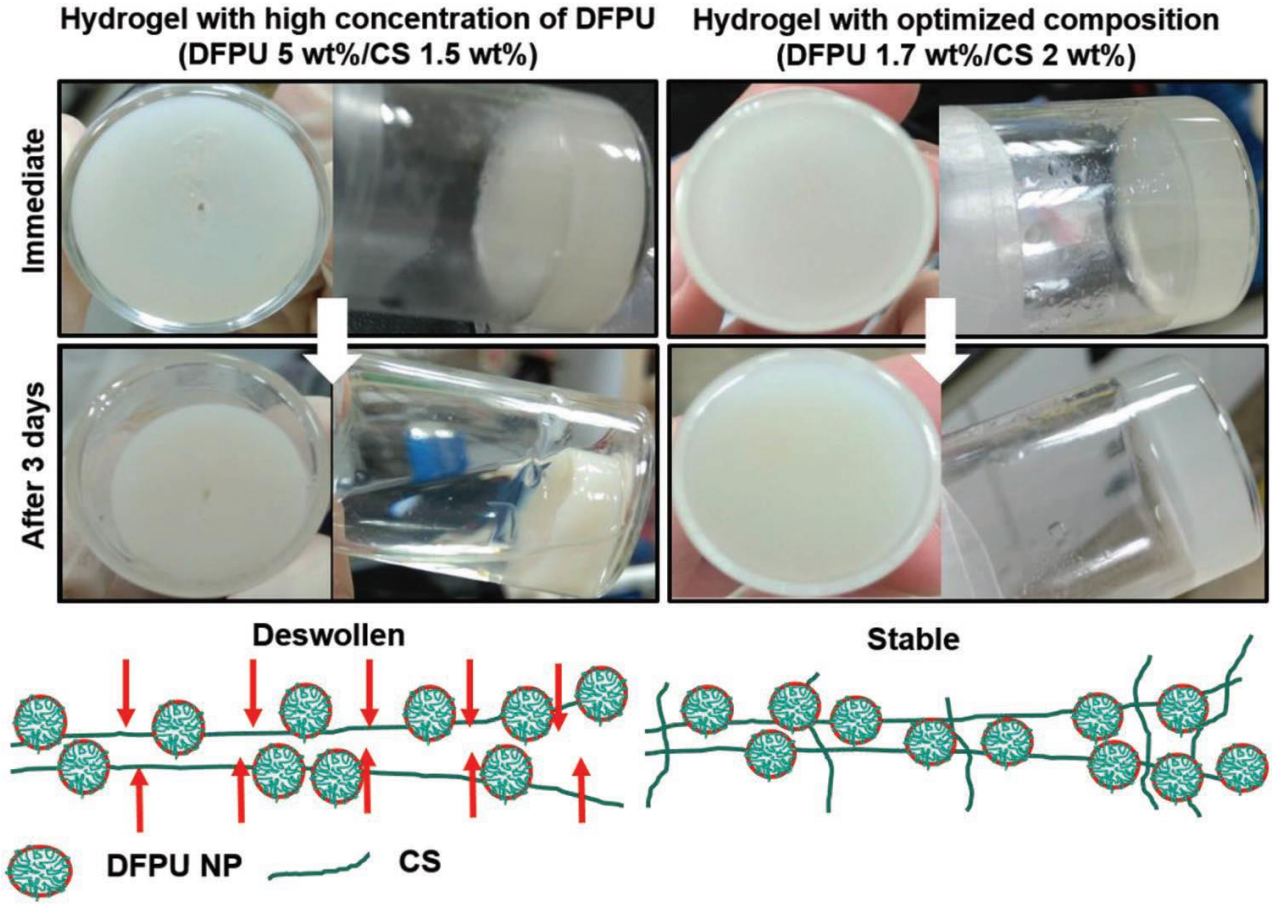
Figure 11. Optimization of the ratio of DFPU nanoparticulate crosslinker and glycol chitosan (DFPU 1.7 wt%/CS 2 wt%) to form stable CS-PU hydrogel without deswelling. A higher ratio of DFPU (DFPU 5 wt%/CS 1.5 wt%) resulted in shrinkage and deswelling (dehydration) of the hydrogel in 3 d.



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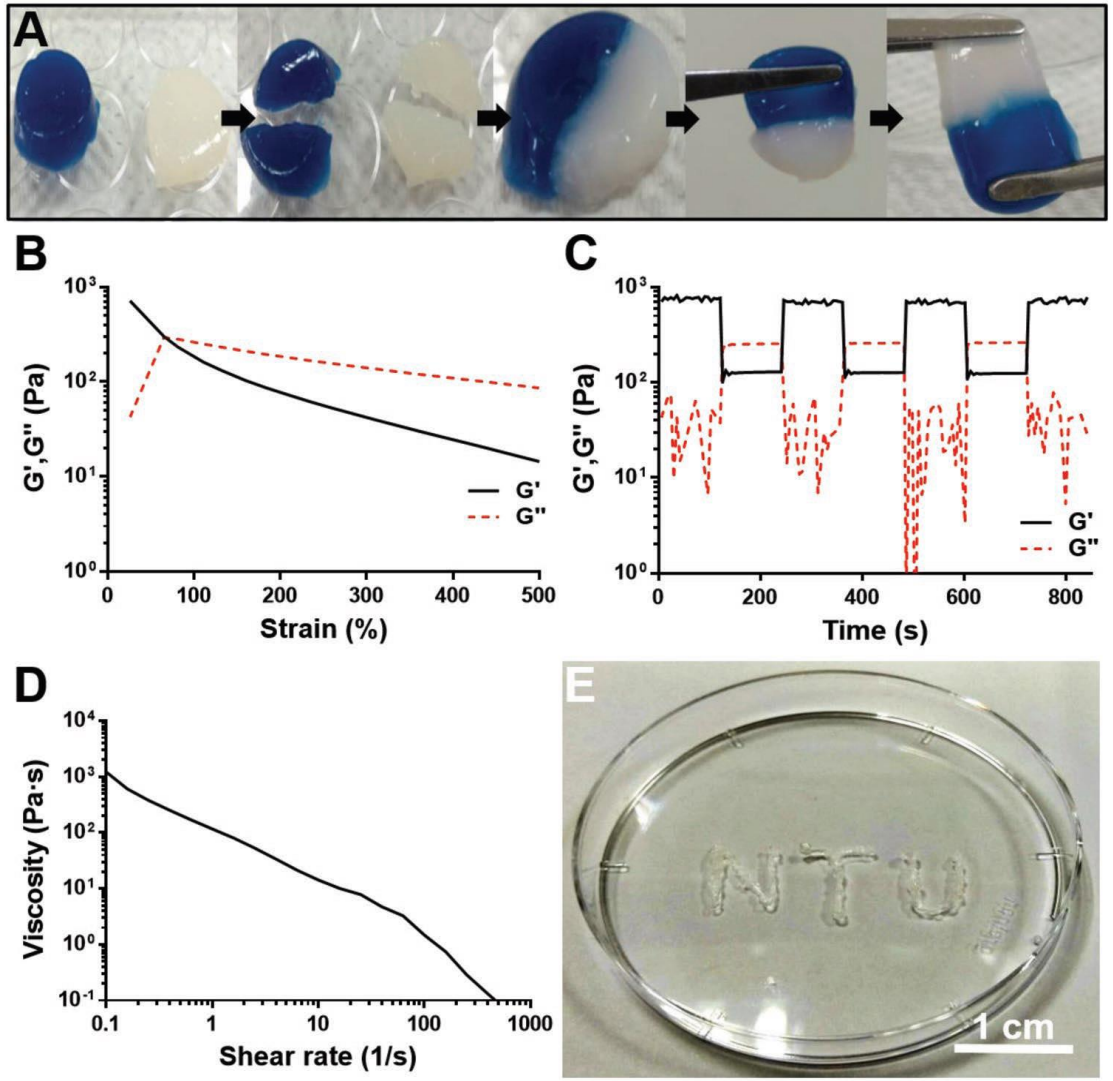

Figure 12. Characteristics of the CS-PU self-healing hydrogel (from DFPU 1.7 wt% and CS 2 wt%). A) Macroscopic hydrogel recovery process. B–D) Rheological properties of the hydrogel. E) Needle injectability. In (A), two circular samples were cut into half and then cross placed together for 5 h. After that, the healed sample was stretched by a pair of tweezers. In (B), the strain for the deconstruction was evaluated by the strain sweep (1–500% strain) experiment at 37 °C and 1 Hz. The gel to sol transition occurred when the strain was ≧ 80%. In (C), the damage-healing properties of hydrogels were demonstrated by measurements under three cycles of the strain change (1% strain → 130% strain → 1% strain → …) at 37 °C and 1 Hz, and the CS-PU hydrogel could restore its structure after high strain-induced structural damage, i.e., with the self-healing capability. In (D), the static shear viscosities of CS-PU self-healing hydrogel versus the shear rate at 37 °C. In (E), the CS-PU self-healing hydrogel could be injected through a 26-gauge needle (260 µm internal diameter).

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- **Self-Healing Hydrogels and Cryogels from Biodegradable Polyurethane Nanoparticle Crosslinked Chitosan**

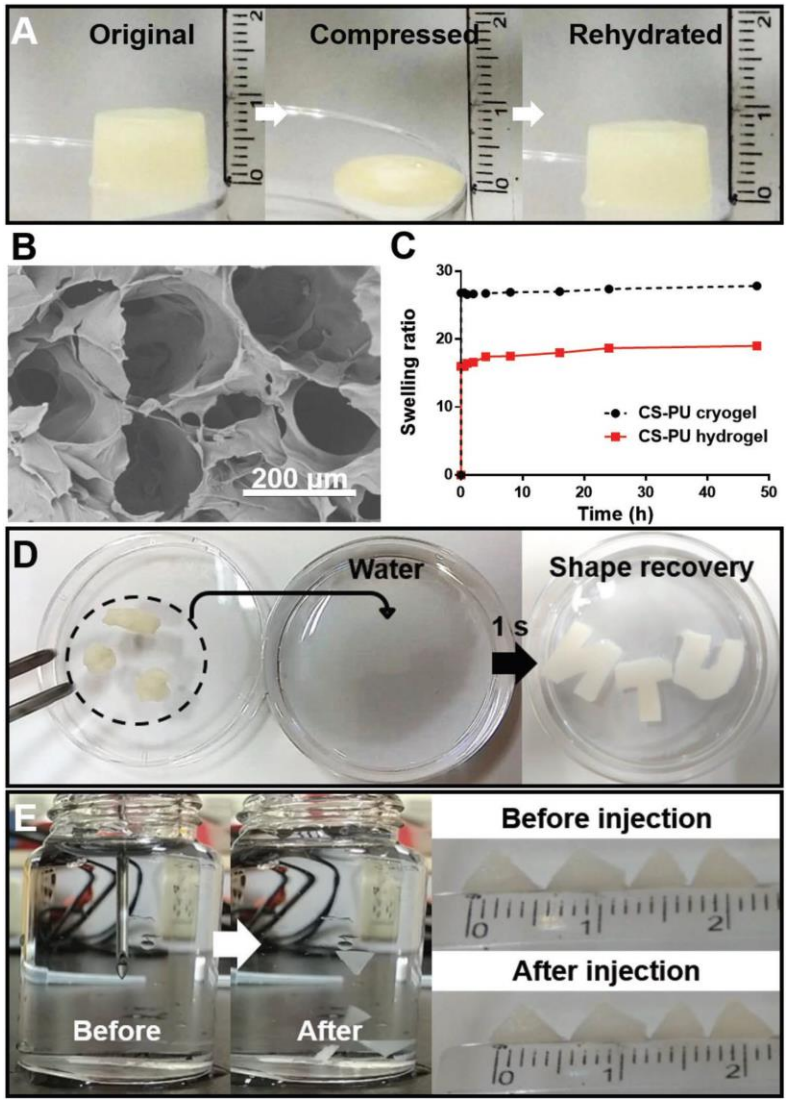

Figure 13. Characteristics of the CS-PU cryogel (from DFPU 1.7 wt% and CS 2 wt%).
A) The compressed cryogel could return to the original shape after rehydration.
B) The SEM cross-sectional image of CS-PU cryogel showing interconnected macroporous network.
C) Water swelling of the lyophilized hydrogels and cryogels, against the immersion time.
D) Cryogels distorted by external force could return to the original shape in 1 s after immersion in water.
E) The cryogel (length 4 mm, thickness 1 mm) could be injected by a conventional 18-gauge needle (838 μm internal diameter) and recover the original shape after injection in water without being distorted.

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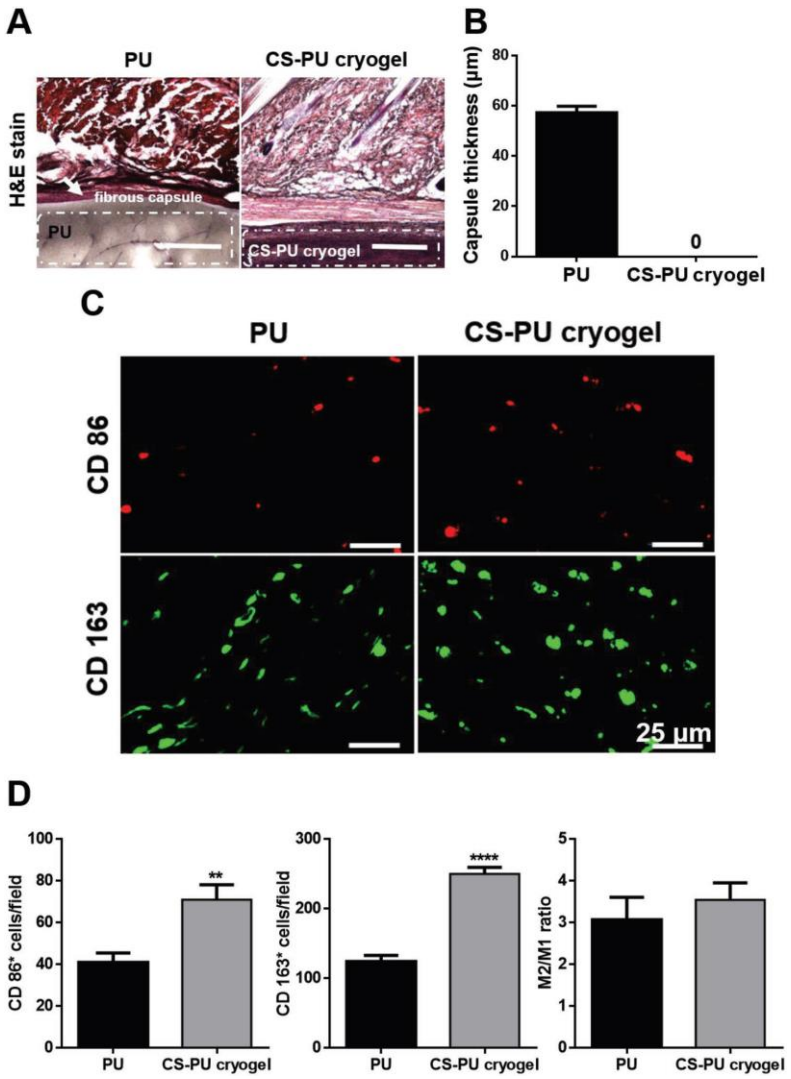

Figure 14. Foreign body reaction of CS-PU cryogels after rat subcutaneous implantation.
A) Histology of H&E-stained sections after implantation for 14 d. The scale bar represents 500 μm.
B) The extent of foreign body reaction could be revealed by the thickness of the fibrous capsule (white arrows) based on the histology.
C) Immunofluorescent images (marker protein expression) of macrophages, stained by the mouse monoclonal anti-CD86 antibody for M1 macrophages (red), or mouse monoclonal anti-CD163 antibody for M2 macrophages (green).
D) Quantification of M1 macrophage and M2 macrophage populations. Results are expressed as mean ± SD, N = 3. **p < 0.01, and **** p < 0.0001 among the indicated groups. PU (nonfunctionalized) films were used as the control.

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