실크 피브로인의 조직공학적 지지체 개발 및 응용

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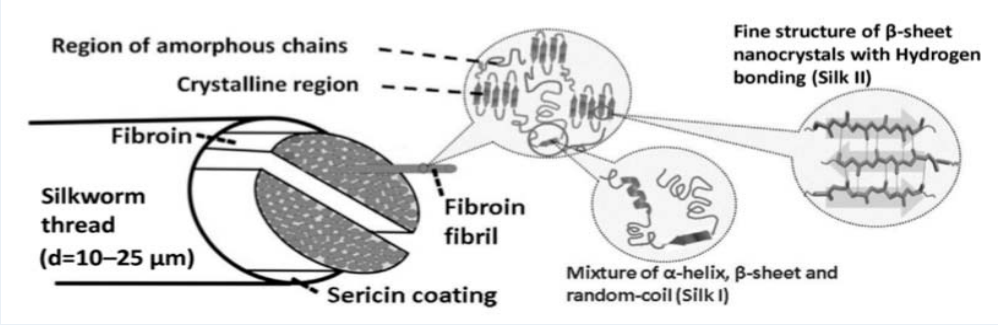
1. 서론

✓ 실크 피브로인(SF; Silk Fibroin)의 조직공학적 지지체 개발 및 응용[1-4]

- 생체 재료로써 실크 피브로인의 물리·화학적 질과 구조:
  (1) 누에고치(silkworm silk)로 부터 추줄되는 실크는 외피를 형성하는 세리신(25%)과 두 가닦의 피브로인(75%) 단백질로 구성 되어 있는 구조.
  (2)실크 단백질은 강력한 수소결합과 결정성 구조를 갖는 섬유산 단백질로 쉽게 물이나 용매에 용해되지 않음.
  (3) 실크 피브로인의 결정구조는 주로 crank나 S zigzag structure 가진 metastable structure의 SF I와 anti- parallel 베타 –sheet structure인 SF II를 가짐.



- 실크 피브로인 가공 방법:
  (1) 다양한 효소를 촉매로 하는 가수분해방법: 가수분해 방법과 연과 과정에 따라 아미노산 분자량이 다름. 분자량 1,050Da
  (2) 단백질의 결합을 끊을 수 있는 유/무기 염, 플르오르화 용매, 이온화 용액, 강산 등을 이용한 방법: 염화칼슘을 이용한 가수분해 방법 시 분자량 65,000Da

- 실크 피브로인의 응용: 당뇨병과 항암제 치료제와 같은 의약품, 식품 및 건강기능 식품, 화장품의 원료에 사용되고 있는 고 부가가치 원료로 널리 알려져 있음.

2. 연구 동향

✓ **Review A Review of Structure Construction of Silk Fibroin Biomaterials from Single Structures to Multi-Level Structure[1]**

- **Structure Design of Silk Fibroin-Based Biomaterials**
  - 세리신을 제거 과정 (Degummed SF)
  - 세리신을 제거 후 염(브롬화 리튬, 염화 칼슘 등) 용액에 넣고 용해 시켜 SF 수용성 용액 제조
  - 이온 제거를 위한 증류수 투석 후 동결 건조

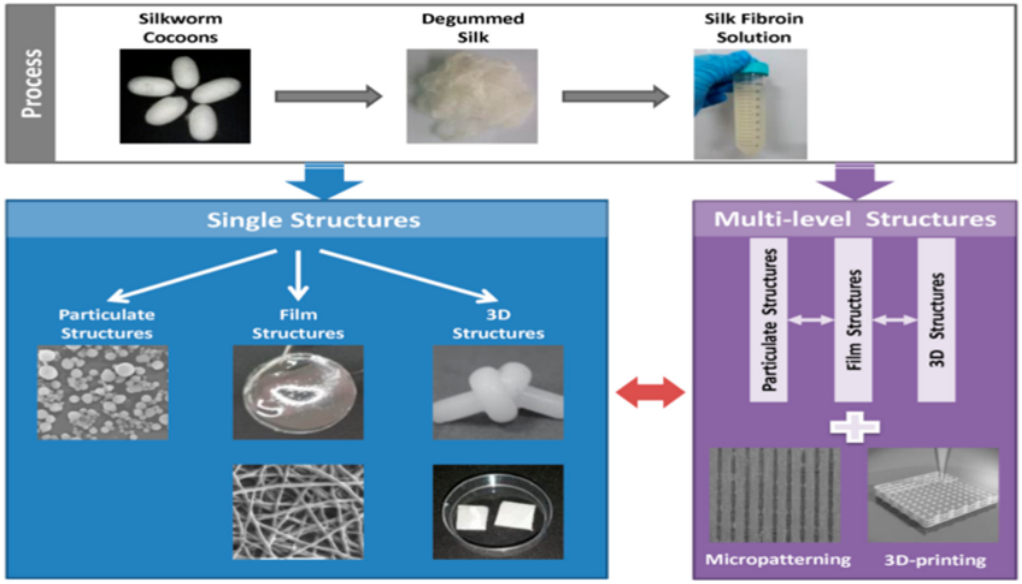


Figure 2. Structural design of SF-based biomaterials from single structures to multi-level structures. Reproduced from [34,35].

- **Single structure**

(1) Particulate Structures

-SF micro-/nanoparticles를 만드는 방법: Self-assembly technology, Desolvation, Spray drying, Laminar jet break-up, caillary microdot, Electrospray techniques, 물리적인 방법에 의한 milling SF 입자 등이 보고되고 있음.

-SF 입자의 응용:Drug delivery, Bioimaging, Therapeutics, Long-term biotracking

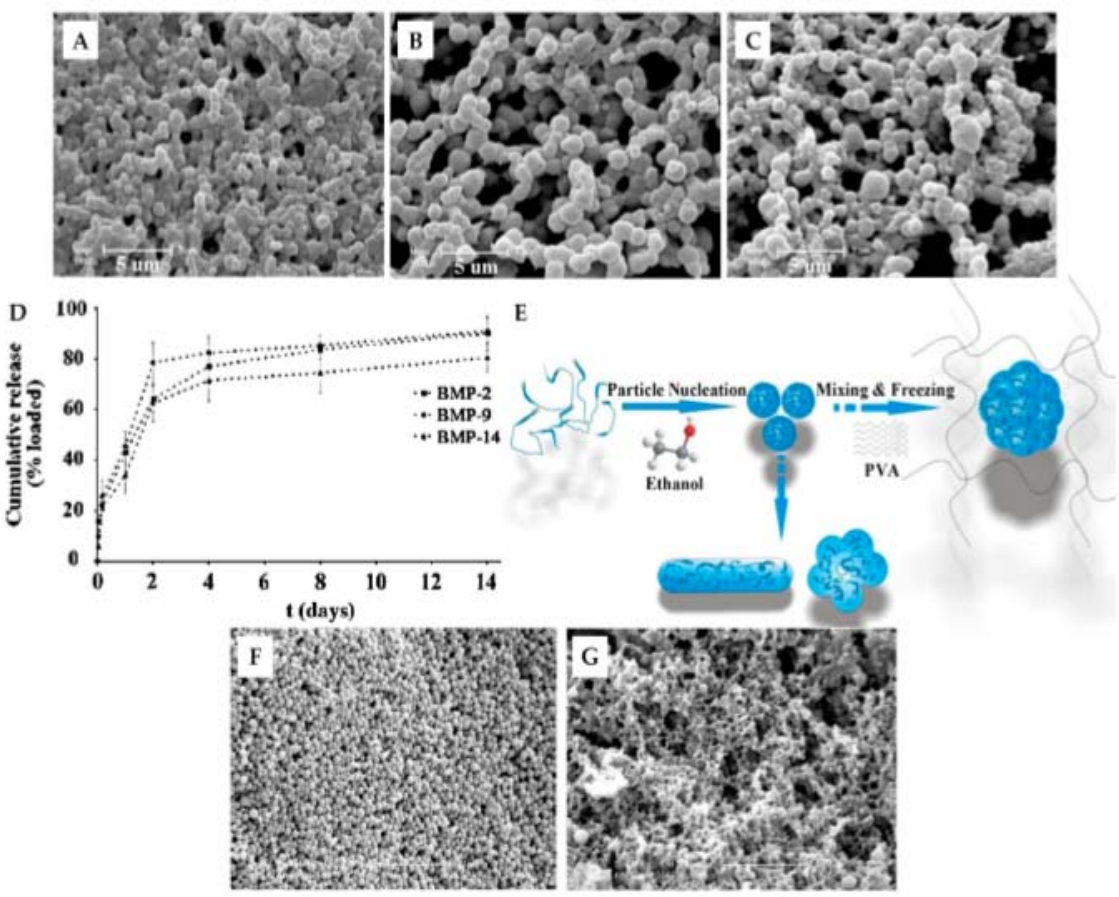


Figure 3. Scanning electron microscope (SEM) images (A–C) of SF microparticles fabricated with different SF: ethanol ratios, (A) 2:1, (B) 3:1 and (C) 4:1, scale bar: 5 μm; (D) Release kinetics of bone morphogenetic protein (BMP)-2, BMP-9, and BMP-14 immobilized in SF particles, with 0.5 μg of BMP per mg of SF. Reproduced from [38]; (E) Mechanism of SF particles' regular formation (F) with addition of poly vinyl alcohol (PVA) and irregular formation (G) without addition of PVA, scale bar: 10 μm. Reproduced from [39].

(2) Film Structures

-SF Film Structures 를 만드는 방법: Casting method, Spin-coating, Deposition, and Spin assisted layer-by-layer assembly, Electrospinning method.

-바이오 소재로써 SF film의 안정성 향상 방법: Stretching, Waterannealing, Slow-drying, Alcohol immersion.

-SF film의 바이오 소재로써의 응용: Artificial skin, Wound dressing, Drug delivery.

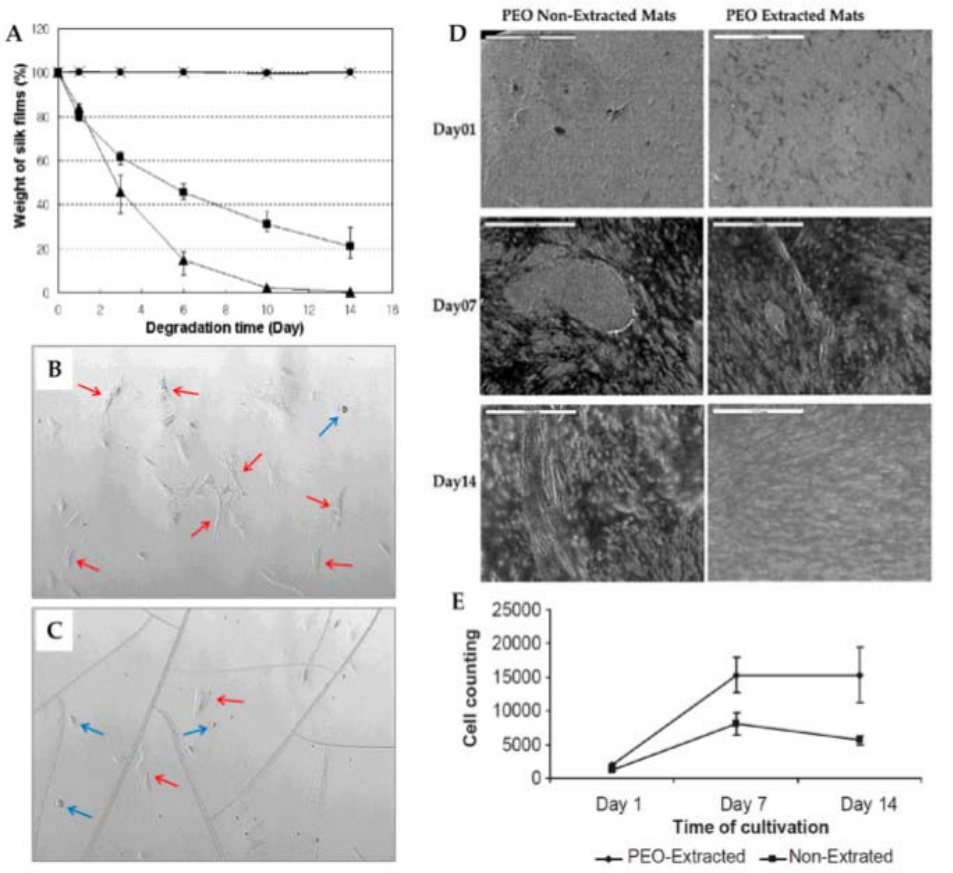


Figure 4. (**A**) Enzymatic degradation of SF films. (▲: SF films by water annealing in enzyme solution; ■: SF films by methanol treatment in enzyme solution; ×: SF films by water annealing in PBS; ●: SF films by methanol treatment in PBS), $n = 5$; Human bone-marrow stromal cells (hMSCs) attachment at 2 h on water-annealed SF film (**B**) and methanol-treated SF film (**C**). The cracks on the film in image (**C**) are induced by methanol treatment. **Red** arrows indicate fully attached cells and **blue** arrows indicate attaching cells. Reproduced from [56]; (**D**) SEM images of bone-marrow stromal cells (BMSCs) growing on polyethylene oxide (PEO) non-extracted and PEO extracted SF mats, respectively, after 1, 7, and 14 days. Scale bar: 500 μm; (**E**) Proliferation of BMSCs grown on SF mats. Seeding density: 2.5 E4 cells/cm^2, $n = 4$. Reproduced from [59].

(3) Three-dimensional Structures

-Three-dimensional structures of SF의 형태: Hydrogels 과 sponge 형태

-SF의 겔화 방법: Sonication, Vortex, Heating, Solvent treatment, Photo-crosslinking, Electrogelation

-겔화의 조절 요소: Temperature, pH, Fibroin Concentration, Addition of other compounds

-Three-dimensional SF sponges의 다공성 구조 제조: Salt leaching, Gas foaming, Freeze-drying

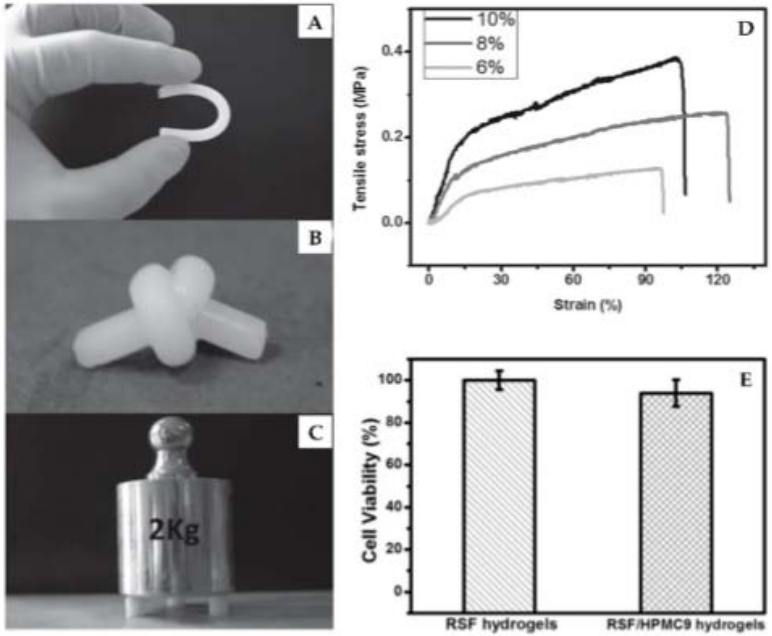


Figure 5. Images of regenerated silk fibroin (RSF)/hydroxypropyl methyl cellulose 9 (HPMC9) hydrogels' reaction to bending (**A**), knotting (**B**) and compressing (**C**); (**D**) Representative tensile curves of RSF/HPMC9 hydrogels with different solid contents; (**E**) Cytotoxicity test of mouse fibroblast cells cultivated with RSF hydrogels and RSF/HPMC9 hydrogels; RSF/HPMCP9: the ratio of RSF to HPMC was 9/1. Reproduced from [34].

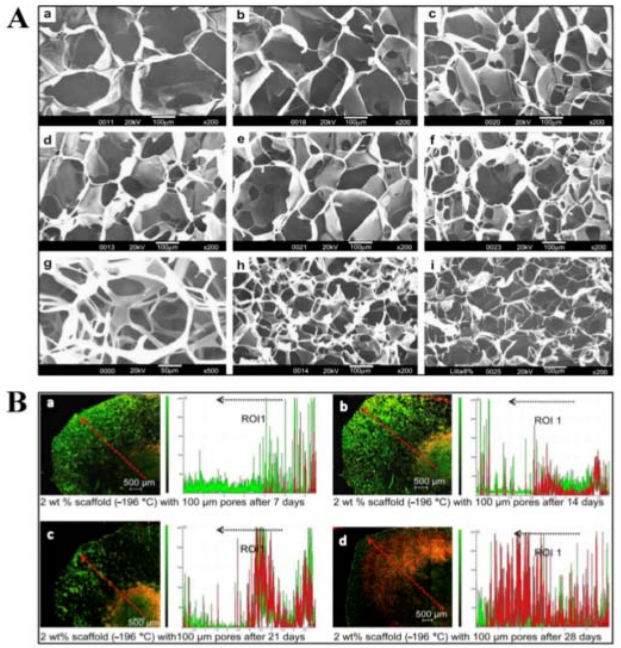


Figure 6. (**A**) Scanning electron microscope images of SF scaffolds fabricated by freeze-drying technique using (**a–c**) 2 wt % SF at −20 °C; (**d–f**) 4 wt % SF at −80 °C; and (**g–i**) 6 wt % SF at −196 °C; scale bar: (**a–f,h,i**): 100 μm; (**g**): 50 μm. (**B**) Confocal laser micrographs of human dermal fibroblast cell migration on SF porous scaffolds fabricated at −196 °C at different time points. The cells are stained with Hoechst 33342 for nuclei (green) and Rhodamine–phalloidin for actin filaments (red); scale bar: 500 μm. The black dotted arrows indicated the region and direction corresponding to the red dotted arrows on the previous graphs; ROI: region of interest. Reproduced from [88].

- **Multi-Level Structure**

(1) Micropatterning Structures

- 바이오 재료의 Micro- 와 nanostructures 제조 방법: Photolithography, Template-assisted electrospray deposition (ESD), Electron beam lithography technique

- Multi-level structures 제조 방법: Photolithography and lyophilization technique , 다양한 micro- and nanostructures의 결합

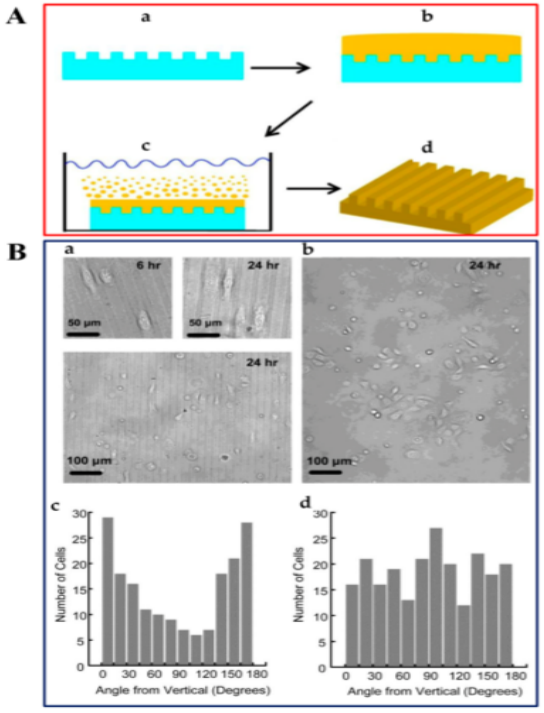


Figure 7. (A) Schematic illustration of the production of patterned SF films: (a) Pre-designed polydimethylsiloxane (PDMS) stamp; (b) Spin-coating SF solution on the PDMS; (c) Extracting the ionic liquid solvent in a methanol bath; (d) Peeling the crystallized patterned SF film from the stamp; (B) Data of cell alignment on patterned SF films as compared to the unpatterned (collagen-coated) films: Optical micrographs of keratinocytes growing on (a) patterned SF films at 6 and 24 h and (b) unpatterned film at 24 h; Histograms of cell alignment on (c) patterned SF films and (d) unpatterned films. The *x*-axis represents cell angle; the *y*-axis represents cell counts. Adapted with permission from [101]. Copyright (2007) American Chemical Society.

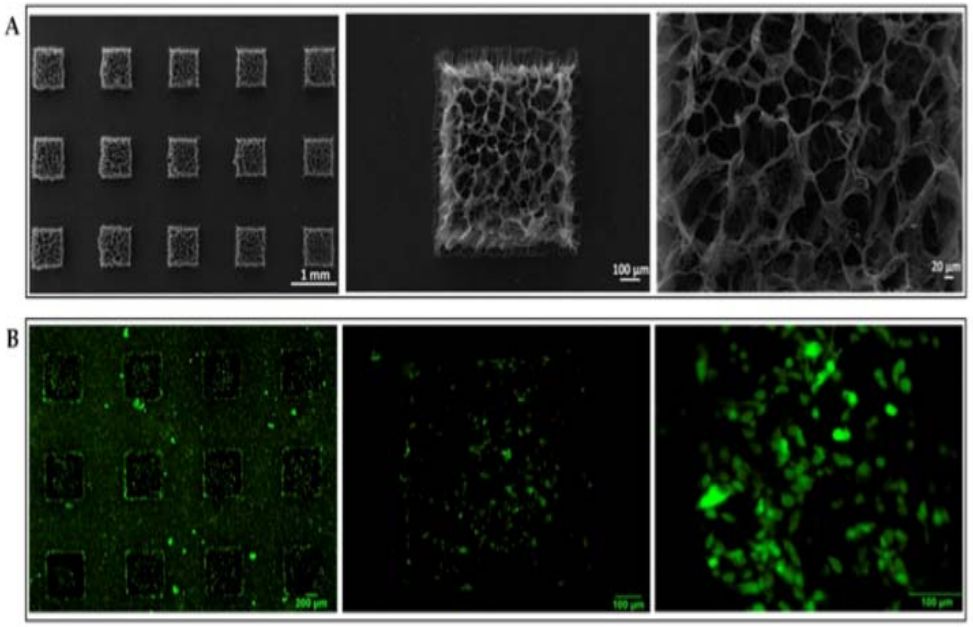


Figure 8. (A) Scanning electron microscope images of micropatterned SF/gelatin methacrylate (GelMA) porous scaffolds; (B) Fluorescence images (2×, 10×, and 20×) of NIH-3T3 fibroblast cells stained with live/dead viability kit after cultured on the micropatterning scaffolds for one day. Reproduced from [108].

(2) Three-Dimensional Printing Structures

- 바이오 재료의 Micro- 와 nanostructures 제조 방법: Photolithography, Template-assisted electrospray deposition (ESD), Electron beam lithography technique
- 3차원 프린팅 구조 제조 방법: 3D bioprinting
- 응용: organs과 tissues

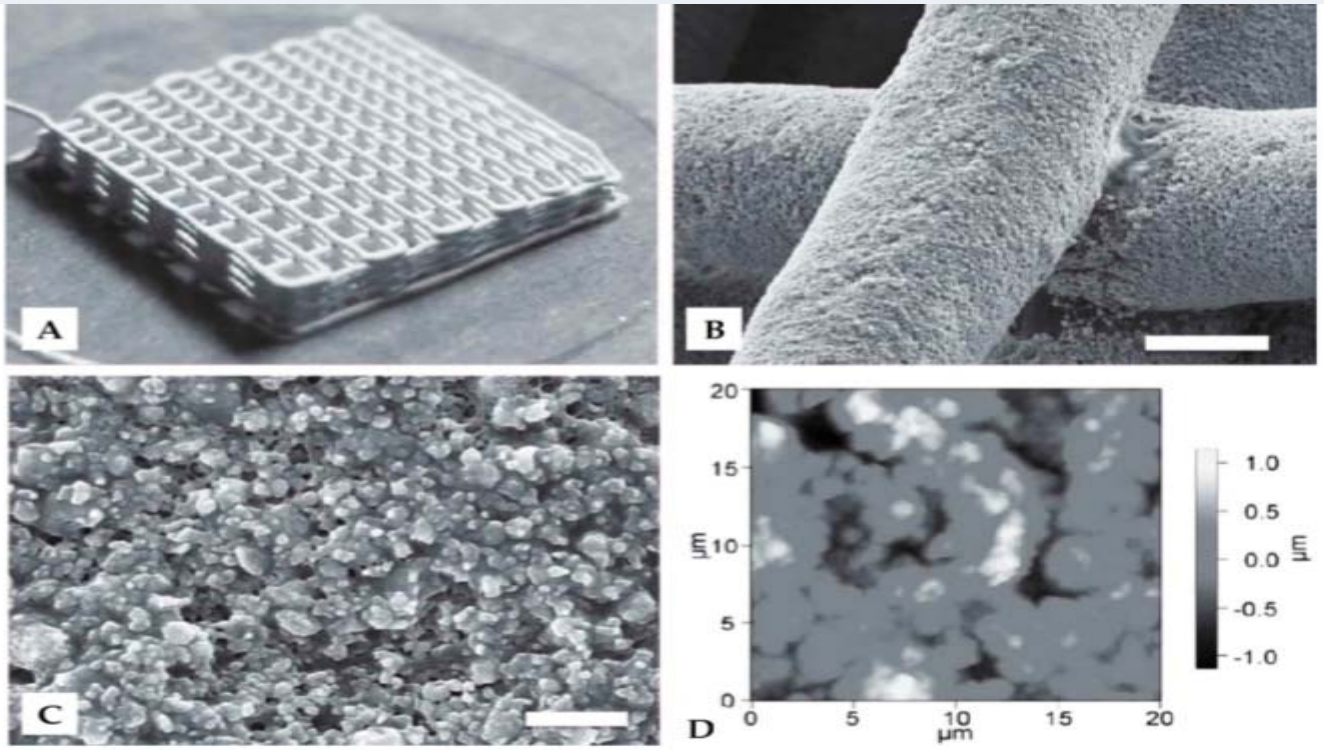


Figure 9. (A) Optical image of three-dimensional printing (3DP) silk/hydroxyapatite (HA) scaffold; (B) Scanning electron microscope image of individual silk/HA filaments at intersection. Scale bar: 100 μm; (C) Higher magnification image of the silk/HA filament surface. Scale bar: 10 μm; (D) Height profile of a representative silk/HA filament observed by atomic force microscopy (AFM). Reproduced from [113].

✓ A Biomimetic Silk Fibroin/Sodium Alginate Composite Scaffold for Soft Tissue Engineerin[2]

- 생체 모방 인공 지지체 재료: 실크 피브로인 (SF) / 알긴산 나트륨 (SA) 복합
- 복합 소재의 다공성 특성: 대부분 공극 직경이 54~532 μm, 공극률이 66~94 %
- 물리적, 화학적으로 SF/SA 복합소재는 적당한 공극 구조 와 향상된 팽창용량은 세포 외 기질에 가까운 생리적 환경을 조성하여 세포 증식을 향상시킴으로써 연조직 공학을 위한 잠재적 후보 물질임을 확인함.

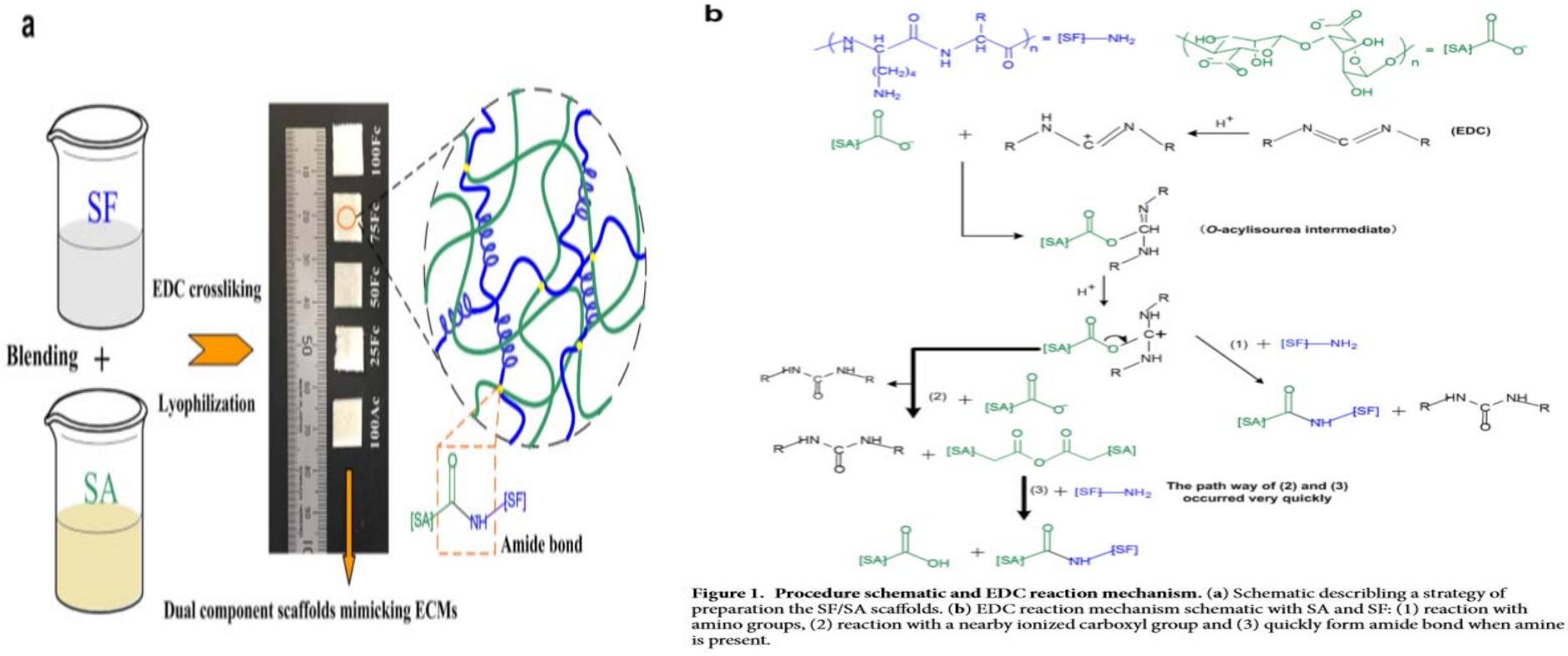


Figure 1. Procedure schematic and EDC reaction mechanism. (a) Schematic describling a strategy of preparation the SF/SA scaffolds. (b) EDC reaction mechanism schematic with SA and SF: (1) reaction with amino groups, (2) reaction with a nearby ionized carboxyl group and (3) quickly form amide bond when amine is present.

✓ Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink[3]

- 바이오 프린팅 방법을 이용한 3 차원 구조으로 세포 성장에 도움이되는 최적화된 새로운 세포 외 매트릭스 (dECM) 바이오 링크 (bioellink) 개발
- 세포 증식, 생존 및 long-term function에 중요한 신호를 입증할만한 능력, 지방, 연골 및 심장 조직 포함한 tissue-specific dECM bioinks 개발
- Bioprinting 방법을 사용하여 인쇄 된 dECM 구조물은 높은 세포 생존력과 기능성을 보고함.

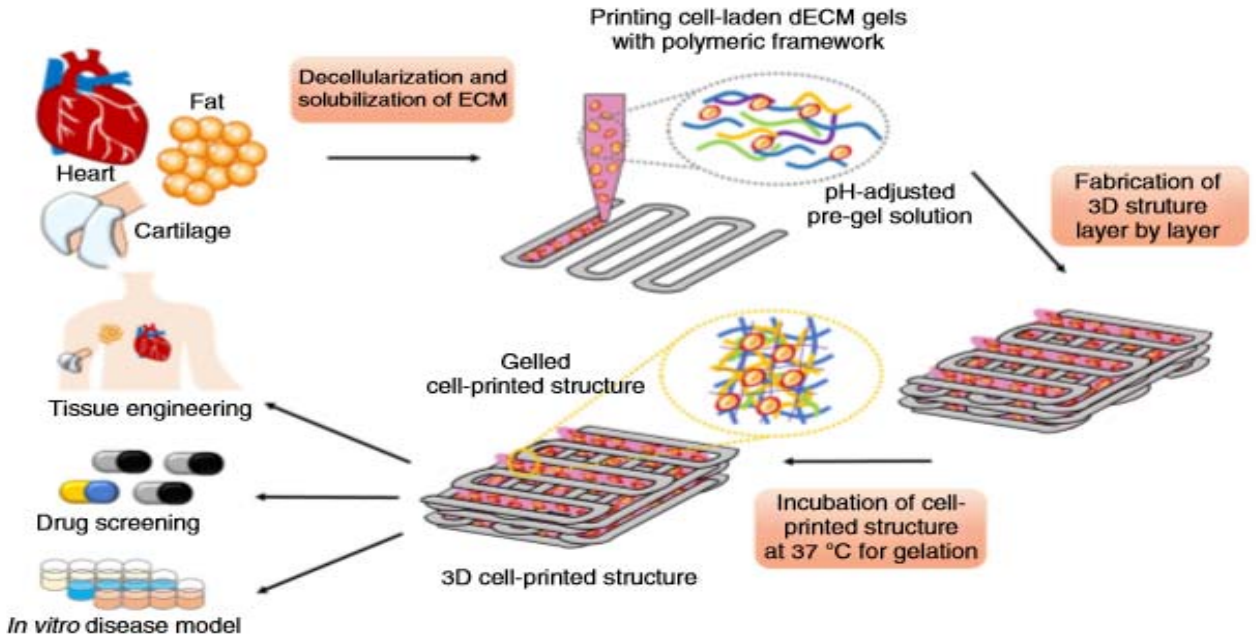


Figure 1 | Schematic elucidating the tissue printing process using dECM bioink. Respective tissues were decellularized after harvesting with a combination of physical, chemical and enzymatic processes, solubilized in acidic condition, and adjusted to physiological pH. Tissue printing was performed with the dECM bioink encapsulating living stem cells via a layer-by-layer approach followed by gelation at 37 °C. The 3D cell-printed structure has applications in various border areas including tissue engineering, *in vitro* drug screening and tissue/cancer model.

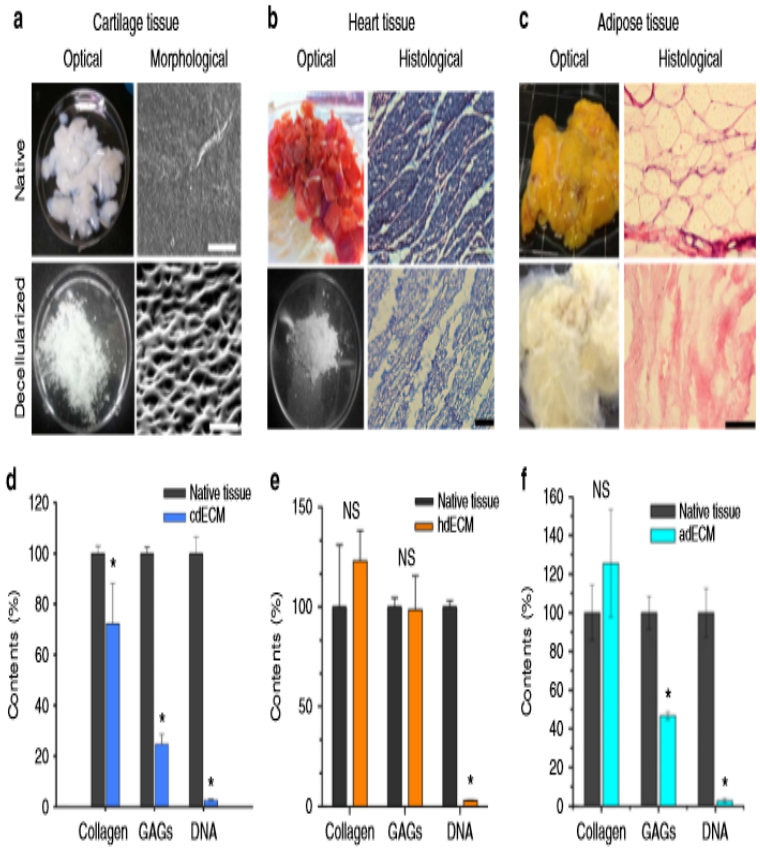


Figure 2 | Decellularization of the native tissues and their biochemical analysis. Optical and microscopic images of native and decellularized (**a**) cartilage tissue (scale bar, 50 μm), (**b**) heart tissue (scale bar, 100 μm), and (**c**) adipose tissue (scale bar, 100 μm). ECM components (Collagen and GAGs) and DNA contents of native and decellularized (**d**) cartilage (cdECM), (**e**) heart (hdECM) and (**f**) adipose (adECM) tissue. All experiments were performed in triplicate. Error bars represent s.d. (*$P<0.05$; NS, no significance).

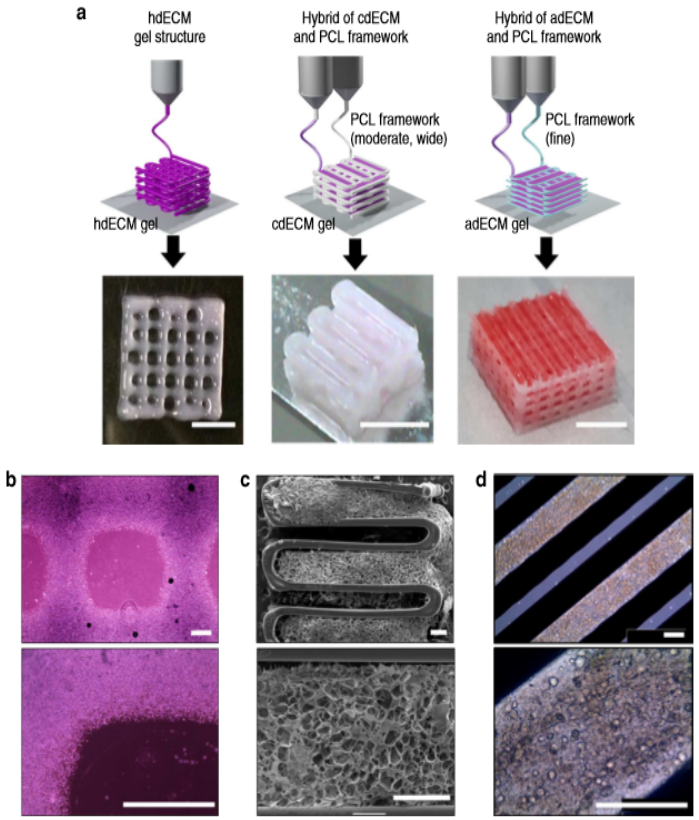


Figure 4 | Printing process of particular tissue constructs with dECM bioink. (**a**) Heart tissue construct was printed with only heart dECM (hdECM). Cartilage and adipose tissues were printed with cartilage dECM (cdECM) and adipose dECM (adECM), respectively, and in combination with PCL framework (scale bar, 5 mm). (**b**) Representative microscopic images of hdECM construct (scale bar, 400 μm), (**c**) s.e.m. images of hybrid structure of cdECM with PCL framework (scale bar, 400 μm) and (**d**) microscopic images of cell-printed structure of adECM with PCL framework (scale bar, 400 μm).

3. 결론

✓ 실크 피브로인의 조직공학적 지지체 개발 및 응용

- 조직공학적 지지체로서의 재료 특성: 생분해성, 생체적합성, 적합한 기계적 물성, 다공성 등.
- 조직공학용 생체재료로 사용되는 천연재료: 다당류와 단백질이 있음.
- 다당류로는 셀룰로오스, 알긴산염, 히알루론산, 전분, 덱스트란, 헤파린, 키틴, 키토산 등.
- 단백질로는 콜라겐, 피브린, 실크 피브로인이 있음.[5]
- 실크 피브로인은 생체적합성이 뛰어 나고 기계적 특성, 분해 속도 조절이 가능하여 바이오 메디컬 응용분야에 조직공학적 연구가 활발하게 이루어지고 있고 향후 중요한 바이오 산업화 가능성을 제시하고 있음.[4]
- 보고된 연구에 의하면 실크 피브로인은 필름, 섬유, 그물 (net), 그물망 (mesh), 막 (membrane), 실 (yarn), 스펀지 (sponge), 하이드로겔 (hydrogel) 등 다양한 형태로 제 조 되어 뼈, 인대,
  연골, 힘줄과 같은 다양한 조직의 재생/공학 기술에 대한 연구가 보고되고 있음.[4]

4. 참고 문헌

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4. 약물전달과 생체 조직공학용 바이오 재료의 소개 및 연구 동향, 류원형, 양다솜, 김현령, 기계저널

5. 조직 재생용 실크 피브로인 생체재료, ReSEAT 분석리포트