

실크 피브로인의 메디컬 의료용 소재 개발 및 응용

Chung-Ang University, Da Vinci College of General Education
OK JA Yoon

1. 서론

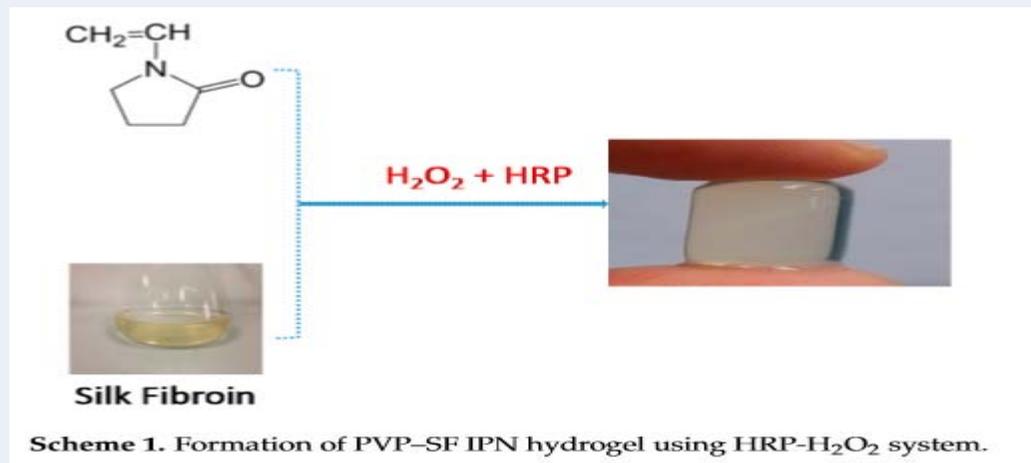
✓ 실크 피브로인의 메디컬 의료용 개발 및 응용^{1, 2}

- 실크 기반의 바이오 메디컬 재료는 수 세기 동안 봉합 재료로 사용.
- 실크 재료는 상처 치료 후에 쉽게 녹고 염증 반응을 일으키지 않음.
- 실크 기반의 바이오 메디컬 재료는 대량 생산이 가능하고 내구성이 뛰어나며 가공 방법이 간단하여 다양한 바이오 메디컬 텍스타일이나 섬유 기반의 임플란트 제조에 주로 사용됨.
- 의료 분야에서 실크와 같은 섬유 활용은 창상 치료, 임플란트 형 수술용 섬유, 카테터 관련 제품, 의료용 부직포 등으로 앞으로 활용도가 높아 전망이 밝음.

2. 연구 동향

✓ Silk Fibroin/Polyvinyl Pyrrolidone Interpenetrating Polymer Network Hydrogels³

- Silk fibroin과 N-vinyl-2-pyrrolidone mixtures의 비율을 조절하여 fibroin hydrogel의 단점인 투명도와 탄성력을 보완한 interpenetrating polymer network(IPN)을 보고함.
- H₂O₂-horseradish peroxidase(HRP) system을 사용하여 fibroin의 random coil 구조가 β-sheet 구조로 변하면서 겔화 과정 시간이 줄어들었음을 확인함.
- 빛의 투과성이 97%이상의 수치를 보여주며, 일주일 동안 90%이상을 유지하였고 기존의 fibroin hydrogel과 비교했을 때, 70%의 압축하에서 95%에 가까운 탄성을 보여줬음. 겔화 과정에서 탄성 계수는 증가 했음.
- 결과적으로 IPN hydrogel이 우수한 기계적 성질 및 우수한 투명성을 갖는다는 것을 보고함.



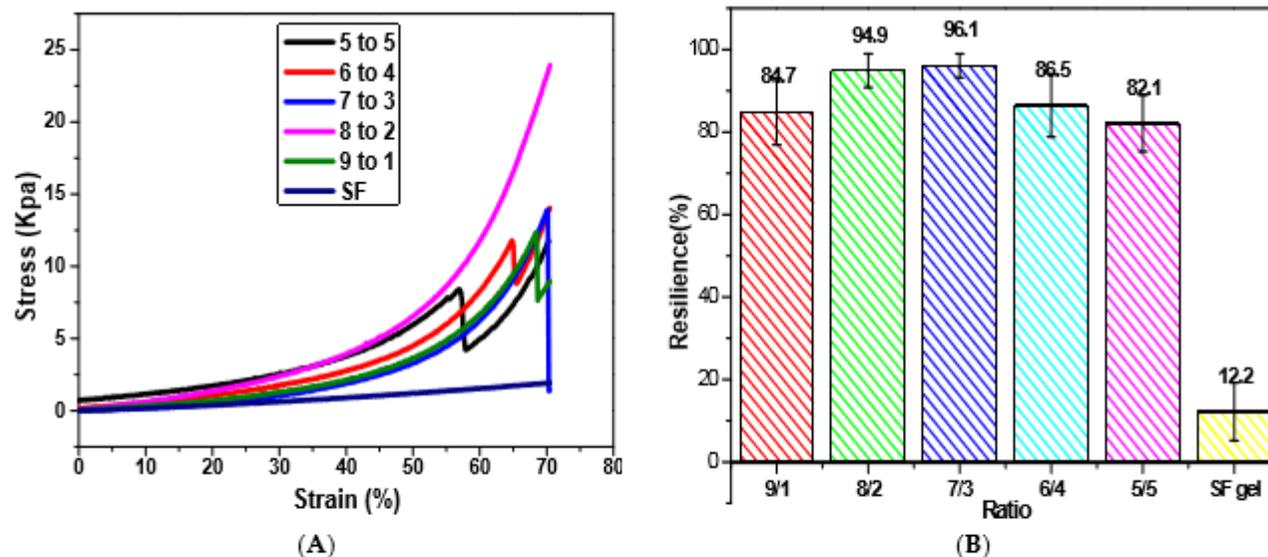


Figure 3. Compression mechanical properties. (A) Stress-strain curves of PVP-SF IPN hydrogels. (B) Stress resilience of PVP-SF IPN hydrogels. The solid content was 10 g/L, the temperature was 25 °C and the relative humidity was 65%.

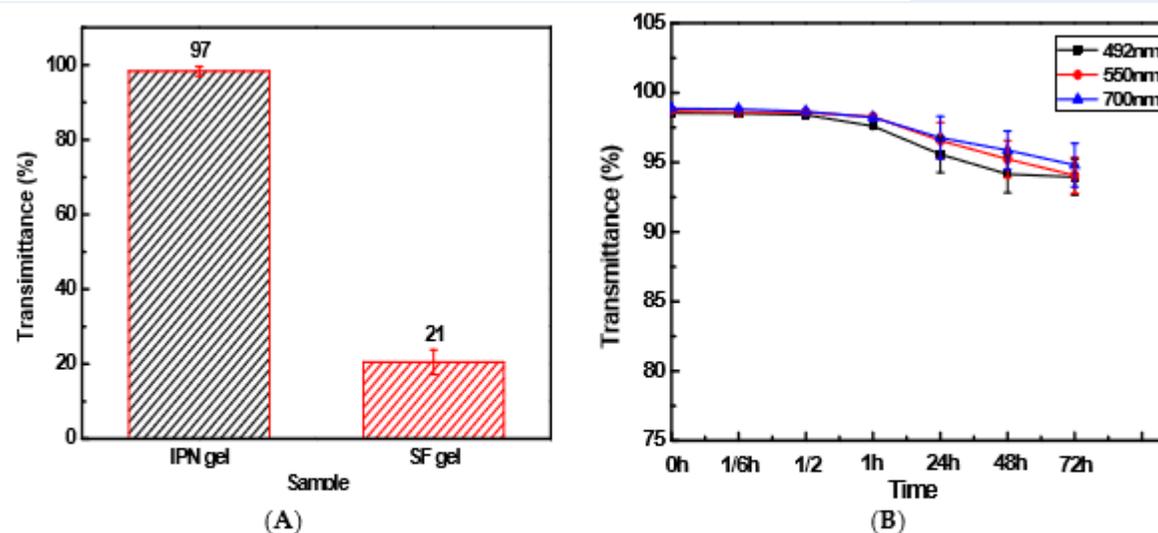


Figure 4. Light transmittance (A) at 550 nm. (B) IPN hydrogel transmittance at different times; The solid content of IPN hydrogel was 10 g/L, SF:NVP was 8:2 and the thickness of material was 4.4 mm.

✓ Effects of silk fibroin in murine dry eye 4

- 이 연구는 안구 건조 모델의 마우스 모델에서 실크 피브로인의 효과를 조사하기 위한 보고서임.
- 12주 이상인 NOD.B10.H2b 쥐에 30~40%의 습도에 노출시켜 scopolamine hydrobromide를 10일 동안 주입함으로써 dry eye mouse model을 만들었고 이 모델을 사용하여 silk fibroin이 건조한 눈에 끼치는 영향을 확인함.
- Phosphate buffered saline(PBS)와 비교했을 때, silk fibroin을 주입한 눈에서 눈물량이 증가했고 각막의 부드러움이 증진됨을 확인 함. Hematoxylin와 eosin 혹은 periodic acid Schiff staining을 이용해서 각막과 결막을 봤을 때, 박리된 각막에선 각막 상피세포가, 결막에선 배상세포가 회복됨을 확인하였음.
- 눈물샘에서 immunohistochemistry을 이용해서 염증 인자를 봤을 때, 저해됨을 볼 수 있음.
- 이러한 결과는 실크 피브로인이 건조한 눈의 실험 모델에서 항 염증 효과를 나타냄을 보여주고 있음.

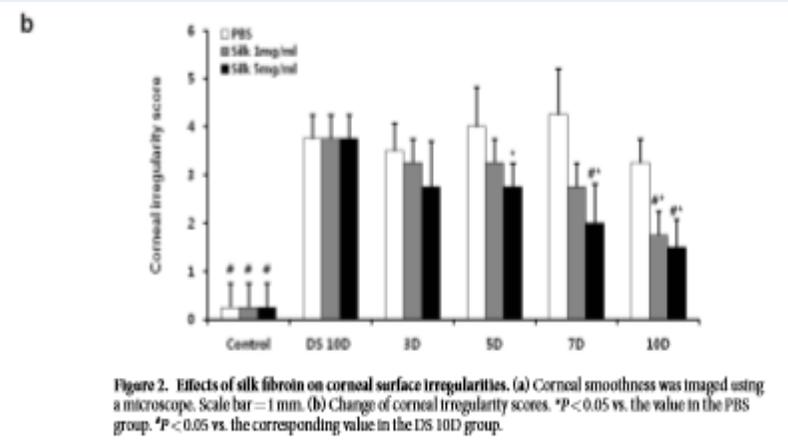
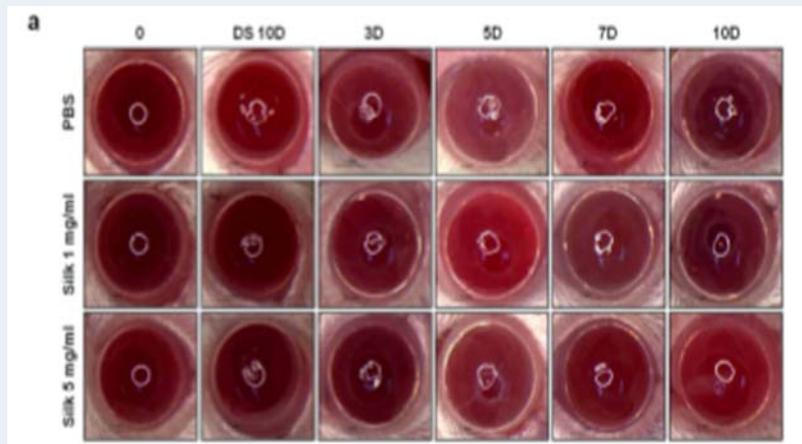


Figure 2. Effects of silk fibroin on corneal surface irregularities. (a) Corneal smoothness was imaged using a microscope. Scale bar = 1 mm. (b) Change of corneal irregularity scores. * $P < 0.05$ vs. the value in the PBS group. # $P < 0.05$ vs. the corresponding value in the DS 10D group.

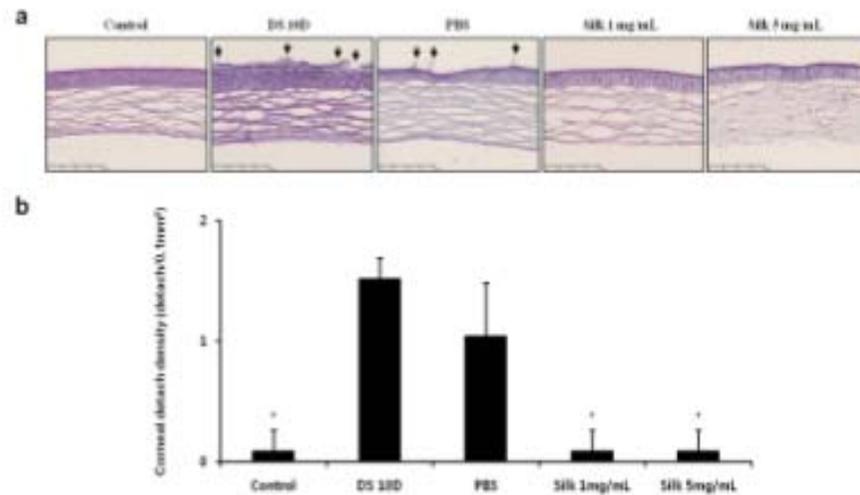


Figure 3. Effect of silk fibroin on detachment of corneal epithelial cells. (a) Hematoxylin and eosin staining. The arrows indicate the detached corneal epithelial cells. Scale bar = 100 μ m. (b) The number of detached corneal epithelial cell. * $P < 0.05$ vs. the corresponding value in the DS 10D group.

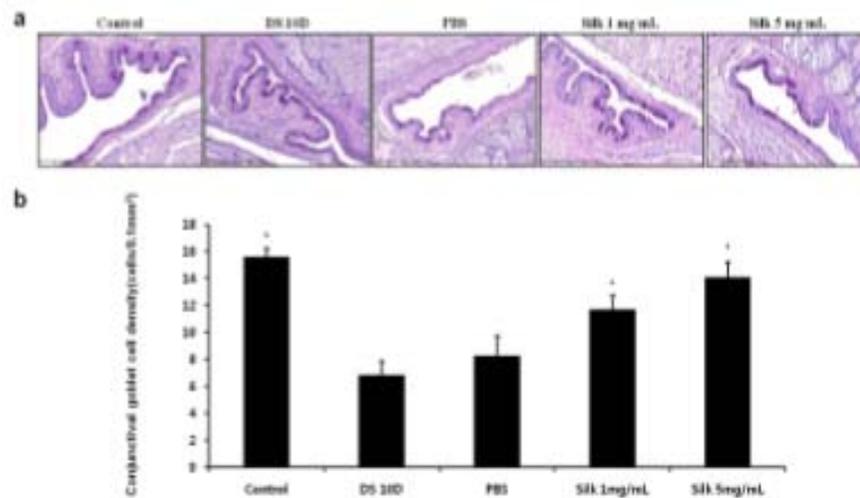


Figure 4. Effect of silk fibroin on conjunctival goblet cell densities. (a) Periodic acid Schiff staining. The stained strong violet color indicate the conjunctival goblet cells. Scale bar = 200 μ m. (b) The number of conjunctival goblet cells. * $P < 0.05$ vs. the value in the DS 10D group.

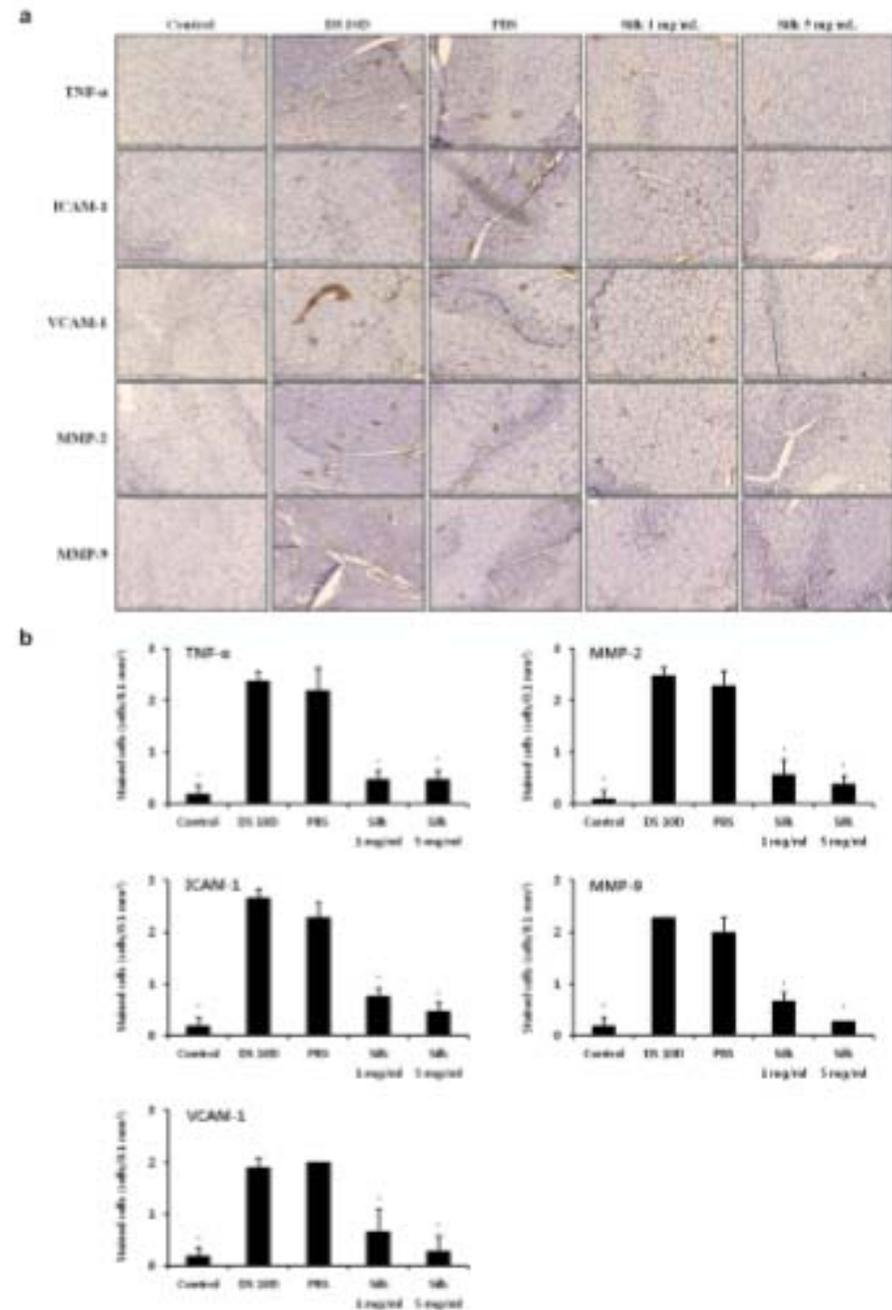


Figure 5. Effect of silk fibroin on inflammation. (a) Immunohistochemistry for inflammatory factors in the lacrimal glands of the mice. Scale bar = 300 μ m. (b) The number of stained cells. * $P < 0.05$ vs. the value in the DS 10D group.

- **Green synthesized nanosilver loaded silk fibroin gel for enhanced wound healing⁵**

- 상피 세포의 성장을 촉진시키는 silk fibroin과 항균제 역할을 하는 silver nanoparticles(AgNPs)를 합친 nanosilver loaded silk fibroin(NSF)를 만들었고 빠른 wound healing 효과를 보고함.
- NSF의 황색포도상구균을 사용한 항균 시험을 통해 항균 효과를 확인하였고, Gel과 합쳐 수컷 wistar 쥐를 이용해 상처 회복 정도를 확인하였고, 시중에 판매되고 있는 topical gel(soframycin gel)과 비교했을 때, 우수한 상처 회복의 특징을 확인하였음.

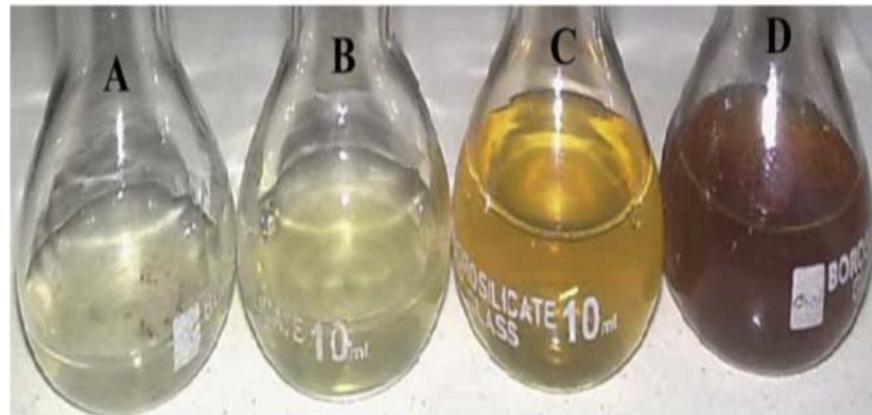


Fig. 1. Optimization of Silk Fibroin and AgNO₃ Concentration A) Silk Fibroin Solution (6%w/v), B) Silk Fibroin (6%w/v) containing 10⁻⁴ M AgNO₃ solution, C) Silk Fibroin (6%w/v) containing 10⁻³ M AgNO₃ solution and D) Silk Fibroin (6%w/v) containing 10⁻² M AgNO₃.

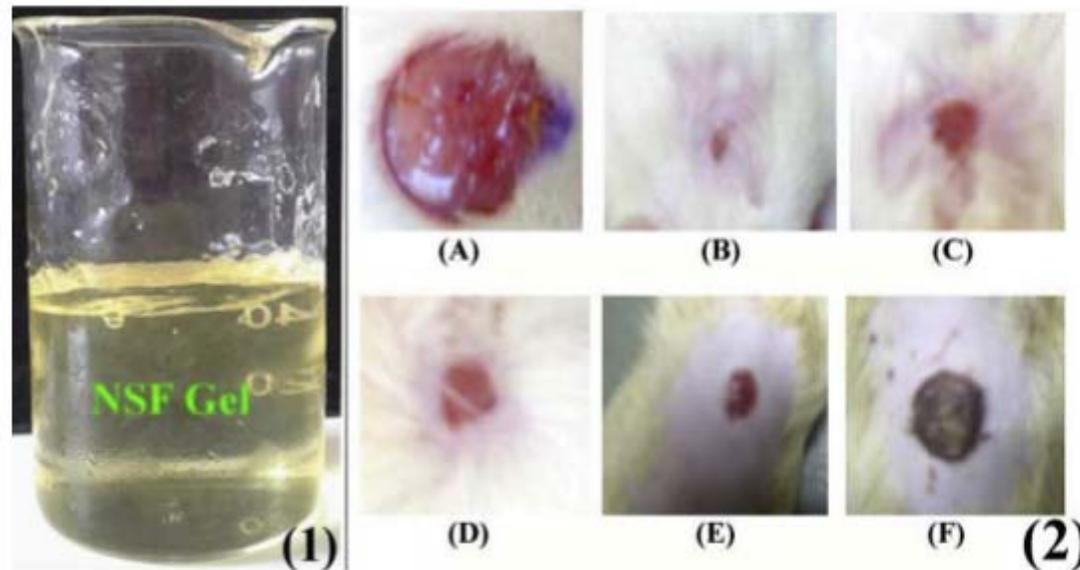


Fig. 6. (1) Digital image of NSF gel. (2) Excision wound healing studies on rats showing (A) Wound on 0 and wound closure on 15th day for gels containing (B) NSF (C) SF (D) SNs (E) Positive control (Soframycin gel) (F) Negative control.

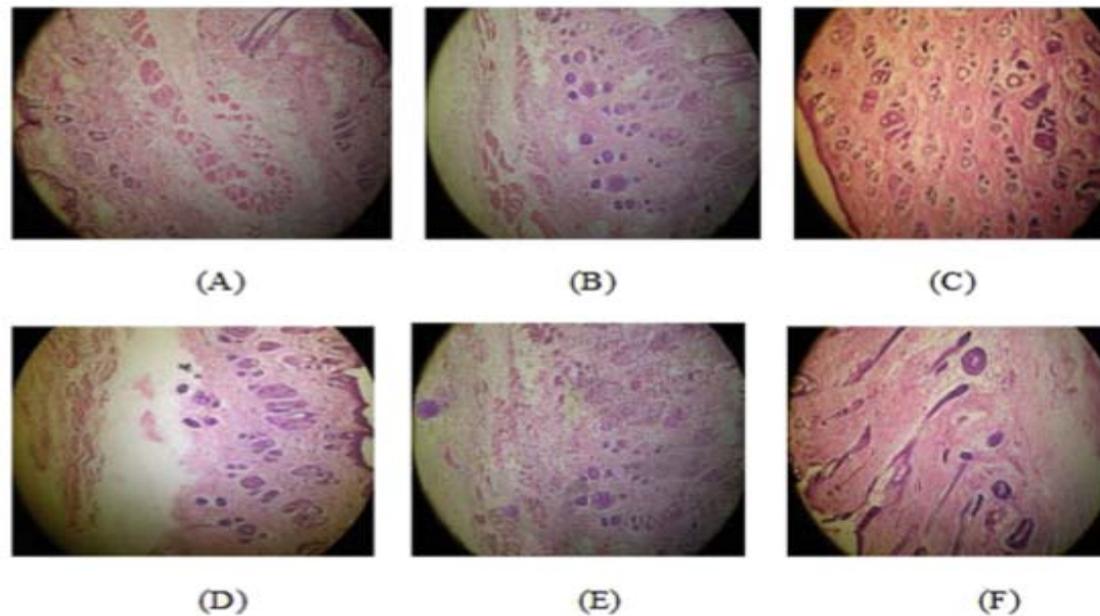


Fig. 7. Histological analysis of H&E stained section of the granulation tissue of different groups treated with (A) NSF, (B) SF, (C) SNs, (D) Plain carbopol gel, (E) Positive control and (F) Negative control on 15th day of wound healing at 100 x magnification.

✓ The Fixation Effect of a Silk Fibroin–Bacterial Cellulose Composite Plate in Segmental Defects of the Zygomatic Arch⁶

- 생분해성을 가진 silk fibroin과 bacterial cellulose(SF-BC)의 혼합하여, 생체적합성을 높은 bioresorbable fixation plate를 만들었음.
- 이를 광대뼈 결함이 생긴 성체 쥐의 한 쪽에만 붙이고, 다른 쪽은 붙이지 않게 함으로써 효과를 확인함.
- micro-computed tomography와 조직 실험을 통해 각각 1, 2, 4, 8주로 관찰한 결과, 붙이지 않은 쪽은 뼈가 자라지 않아서 치료되지 않았을 뿐만 아니라, 괴사된 것을 확인함.
- 반면, SF-BC composite plate를 붙인 쪽은 8주라는 짧은 시간 안에, 새로운 뼈가 생겨 완전히 치료가 되었음을 확인함.



Figure 1. Rat model with a segmental defect created in the midzygomatic arch. A, The rat skull is visible. B, A 2-mm bony segment was removed with a medical bone cutter. C, The segment was fixed with a silk fibroin–bacterial cellulose composite plate.

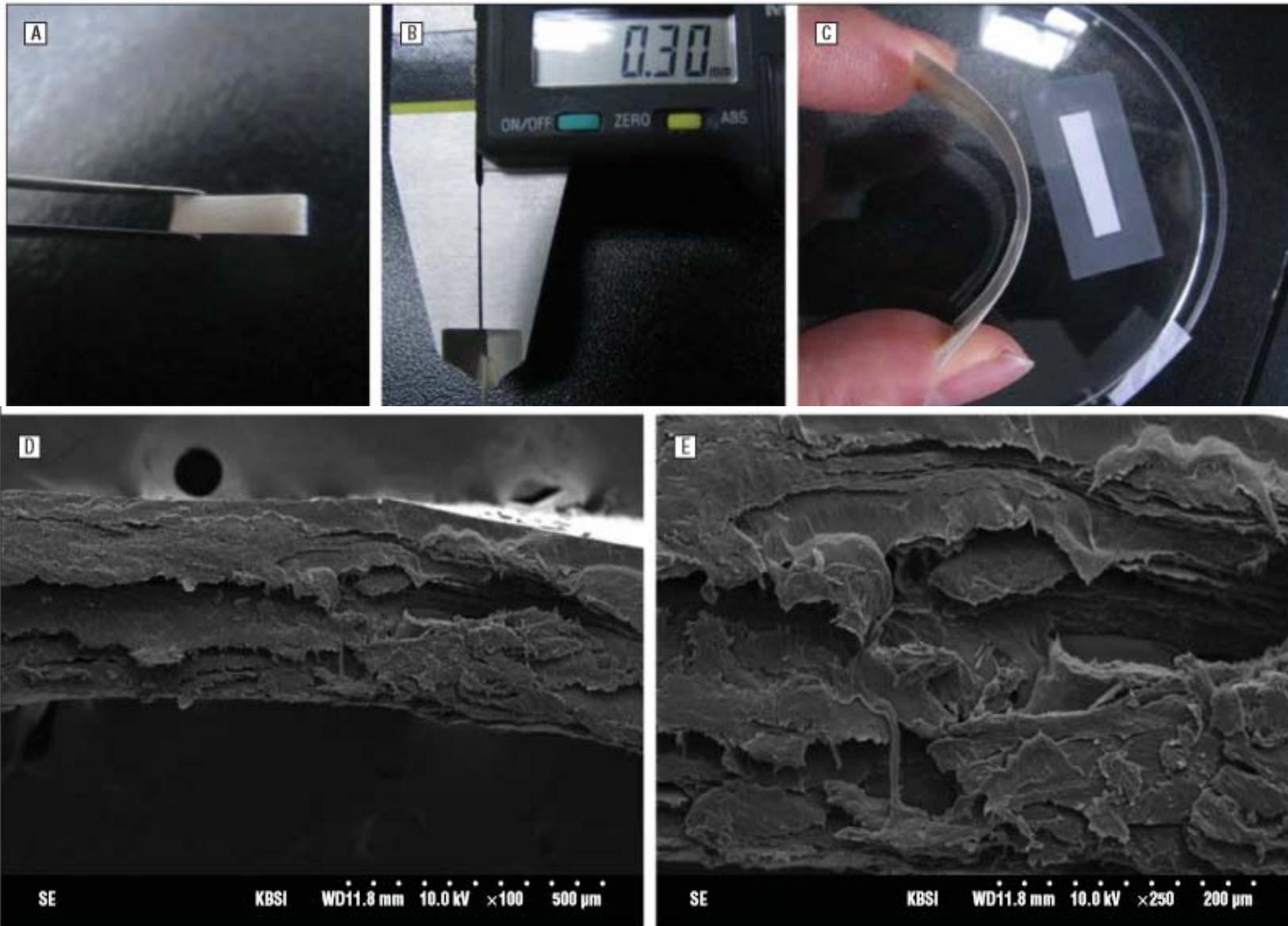


Figure 2. Properties of the flexible silk fibroin–bacterial cellulose composite plate. A, External appearance. B, Thickness measures 300 μm . C, Flexibility. D and E, Scanning electron microscopic imaging identifies no pore structure (original magnification $\times 100$ and $\times 250$, respectively). KBSI indicates Korea Basic Science Institute; SE, secondary electron; and WD, working distance.

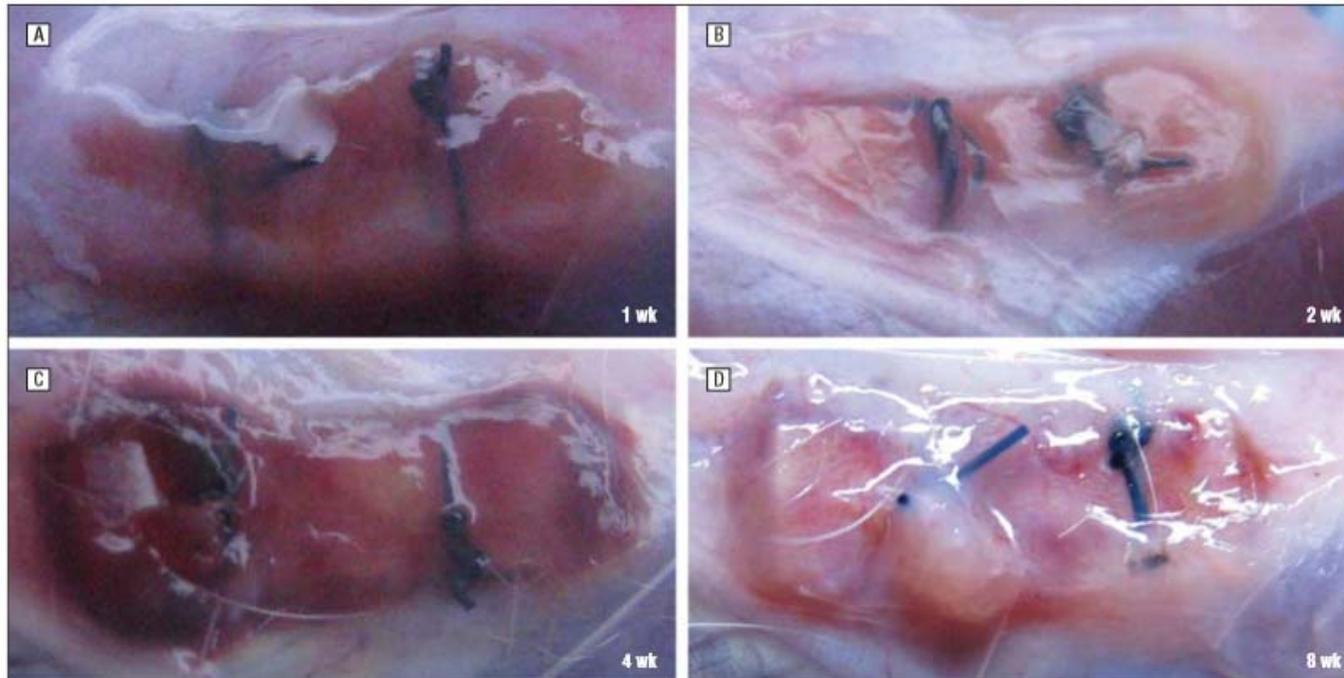


Figure 3. Gross appearances of segmental defects fixed with silk fibroin-bacterial cellulose composite plates at 1, 2, 4, and 8 weeks. No evidence of wound infection, hematoma, or ser

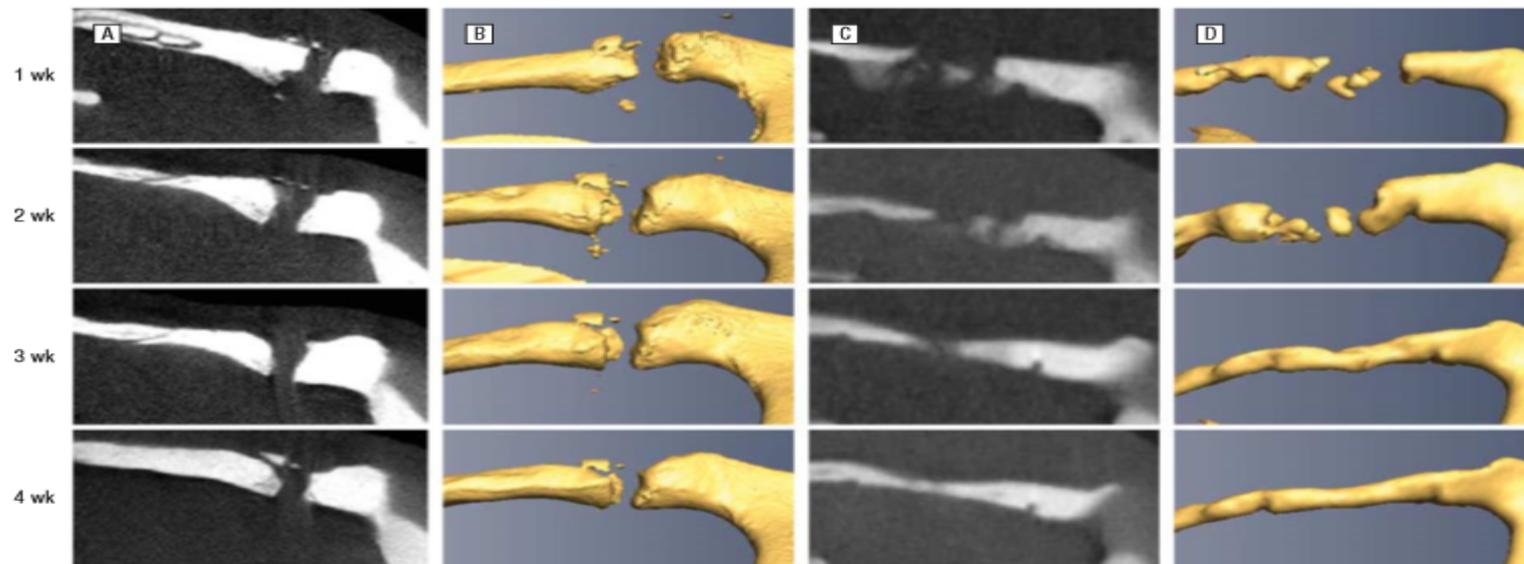
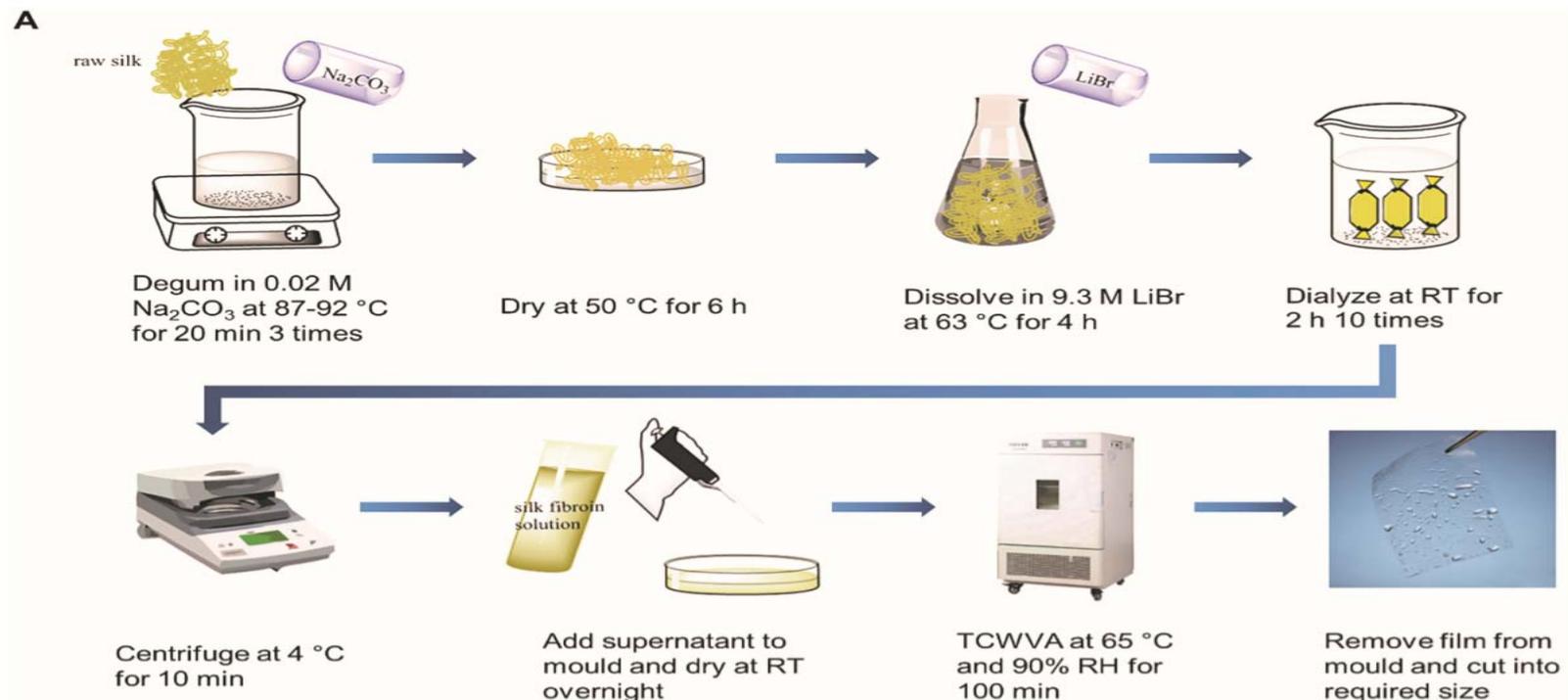


Figure 4. Two- and 3-dimensional micro-computed tomographic images demonstrating the bony healing process during the first 4 postoperative weeks. A and B, No bony regeneration occurred on the control side. C and D, Formation of a hard callus, new bone growth, and complete ossification appeared sequentially on the silk fibroin-bacterial cellulose composite plate side.

✓ **Silk fibroin biomaterial shows safe and effective wound healing in animal models and a randomized controlled clinical trial⁷**

- Silk fibroin의 단점을 보완하기 위하여 green chemistry 방법을 이용하여 투과율 (transmittance), fluid handling capacity, 수증기 투과도 (moisture vapor permeability), 방수성 (waterproofness), bacterial barrier properties, and 생체 적합성 (biocompatibility) 같은 뚜렷한 특징을 가진 필름을 제조함.
- 상처낸 토끼의 두꺼운 조직에 붙여 상처 회복 시간이 줄어들었음을 확인하였고 잘 상처가 잘 치료된 것을 보고함.
- 돼지 모델을 통해 안전성과 효용성을 확인하였고 71명의 환자들을 대상으로 blind test 를 통해 한 번 더 상처 회복 시간과 부작용이 줄어들었음을 보고함.



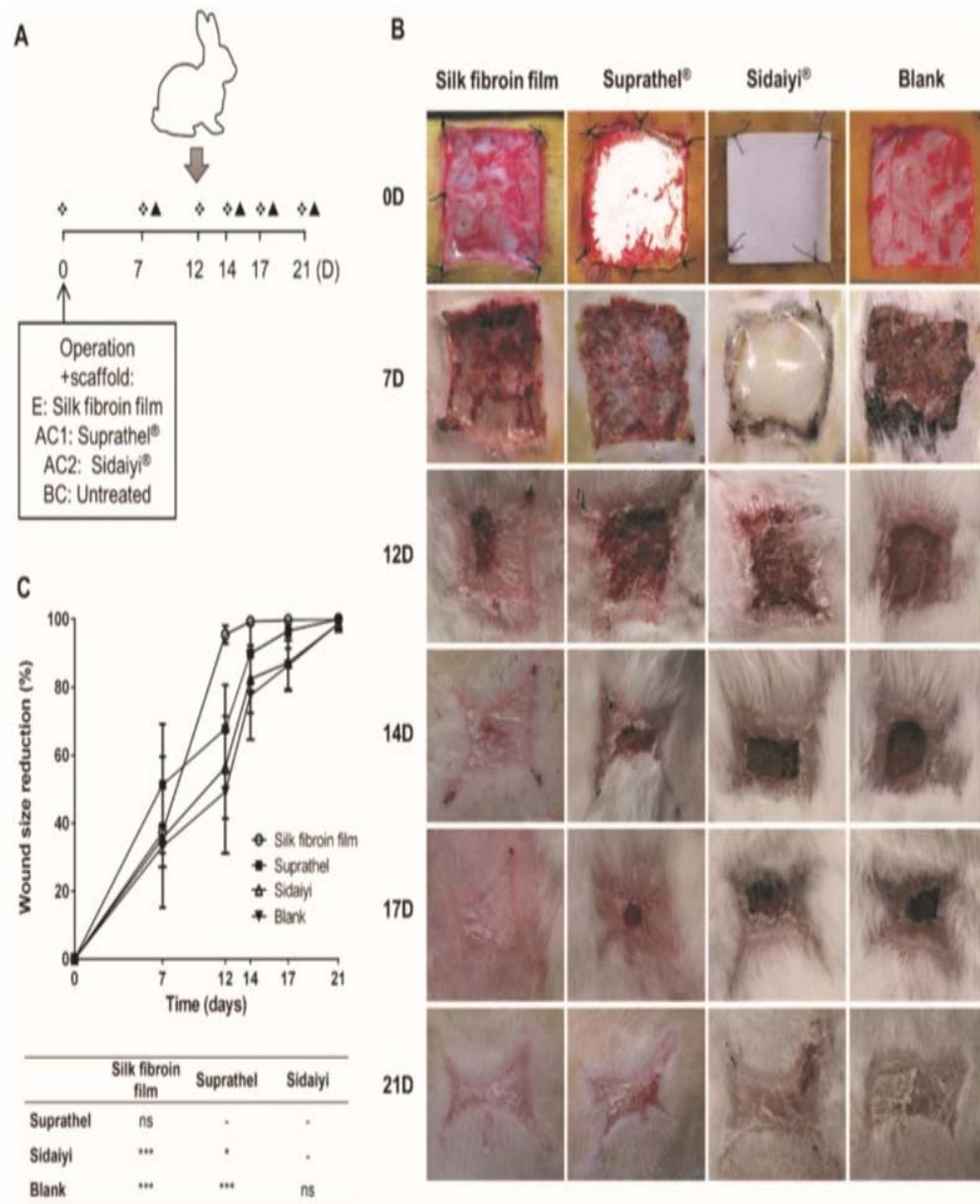


Figure 4. Rabbit model: study design and macroscopic evaluation. A) Full-thickness skin defects were created and left untreated (blank control), or covered with the silk fibroin film (experimental), or with Suprathel (active comparator 1), or with Sidaiyi (active comparator 2). Macroscopic evaluation (◇) was performed on days 0, 7, 12, 14, 17, and 21 postoperation, and histological evaluation (▲) was performed on days 7, 14, 17, and 21 postoperation. B) Gross morphology of the wound healing process within 21 d. C) Percentage of wound size reduction on days 0, 7, 12, 14, 17, and 21 postoperation. Two-way ANOVA was performed to compare between groups. E, experimental; AC1, active comparator 1; AC2, active comparator 2; BC, blank control. ns indicates no significant difference. * $p < 0.05$ and *** $p < 0.001$.

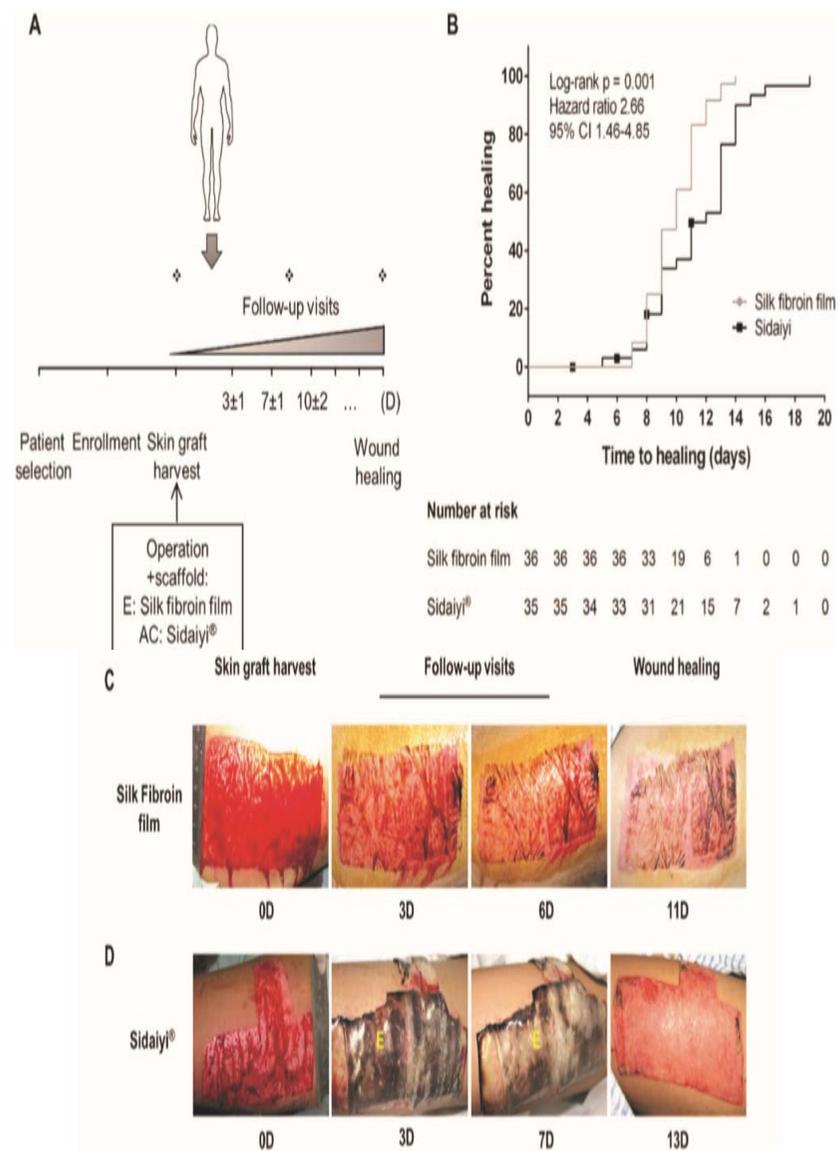


Figure 7. Clinical trial: study design and follow-up evaluation. A) All patients were selected according to the inclusion/exclusion criteria and 71 eligible patients were enrolled for the clinical trial. After harvesting the skin graft, the donor site wounds were covered with the silk fibroin film or with Sidaiyi. Follow-up visits were performed on days 3 ± 1 , 7 ± 1 , 10 ± 2 until the wound was completely healed. Macroscopic evaluation (◇) was performed after skin graft harvest, during the follow-ups and when the wound healed. B) Kaplan-Meier curves comparing cumulative healing by treatment group. C) The representative case from the silk fibroin film group. Macroscopic images were captured after skin graft harvest (day 0), at the 1st follow-up (day 3), at the 2nd follow-up (day 6), and when the wound healed (day 11). D) The representative case from the Sidaiyi group. Macroscopic images were captured after skin graft harvest (day 0), at the 1st follow-up (day 3), at the 2nd follow-up (day 7), and when the wound healed (day 13). E, exudates.

3. 결론

- ✓ 실크 피브로인과 같은 메디컬 섬유 기술은 인체 치료용 메디컬 섬유제품 중 인공혈관, 혈액 성분 분리 필터, 혈액 투석 필터, 수술 용 메시, 창상 피복재, 봉합사 등에 해당하는 세계 시장 규모는 약 156억불에 달하는 것으로 보고되고 있음.²
- ✓ 실크 피브로인과 같은 메디컬 섬유 소재 개발을 위한 주요 핵심 기술²:
 - 의료용 섬유의 주요 소재인 천연 고분자 및 합성 고분자 확보 필요
 - 제사(製絲) 기술
 - 생분해성(biodegradable) 혹은 생체불활성(bio-inert) 3차원
 - 섬유구조 설계 기술 : 직물, 편물, 부직포, 브레이드 형상 등
 - 인체 내·외부에서 시간에 따른 변화까지 제어되는 4차원적 섬유구조 제어기술
 - 생체기능성 및 생체적합성 부여를 위한 화학 물리적 가공 기술
 - 청정생산기술
 - 생체적합성이 요구되므로 임상학적 연구 수반
 - 동물 실험/전임상/임상 기술, 의료용 적합성 평가기술

4. 참고 문헌

1. 바이오메디컬 용도의 섬유, AATCC News (2017.9.12)
2. D. Y. Lim and E. K. Choe, Recent Development Trend on Medical Devices Based on 3-Dimensional Textile Structure, *Biomaterials Research*, 12, 64 (2008)
3. D. K. F. Wu, Z. Yin, T. Zhu, T. Xing, S. C. Kundu and S. Lu, Silk Fibroin/Polyvinyl Pyrrolidone Interpenetrating Polymer Network Hydrogels, *Polymers*, 10, 15(2018)
4. C. E. Kim, J. H. Lee, Y. K. Yeon, C. H. Park and J. W. Yan, Effects of silk fibroin in murine dry eye, *Scientific Reports*, 7, 44364(2017)
5. S. Patil, T. George, K. Mahadik, Green synthesized nanosilver loaded silk fibroin gel for enhanced wound healing, *Journal of Drug Delivery Science and Technology*, 30, 30(2015)
6. J. M. Lee, J. H. Kim, O. J. Lee, C. H. Park, The Fixation Effect of a Silk Fibroin–Bacterial Cellulose Composite Plate in Segmental Defects of the Zygomatic Arch, *JAMA OTOLARYNGOL HEAD NECK SURG*, 139, 629(2013)
7. W. Zhang, L. Chen, J. Chen, L. Wang, X. Gui, J. Ran, G. Xu, H. Zhao, M. Zeng, J. Ji, L. Qian, J. Zhou, H. Ouyang, and X. Zo, Silk Fibroin Biomaterial Shows Safe and Effective Wound Healing in Animal Models and a Randomized Controlled Clinical Trial, *Adv. Healthcare Mater.*, 6, 170012(2017)