ARTICLE

Supporting Information

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

Pressure-Driven Spreadable Deferoxamine -laden Hydrogels for Vascularized Skin Flaps

Lijun Wu,‡[a,b] Suyue Gao,‡[b,c] Tianlan Zhao,‡[b] Kai Tian,[b] Tingyu Zheng,[b] Xiaoyi Zhang,[a] Liying Xiao,[a] Zhaozhao Ding,[a] Qiang Lu*[a] and David L Kaplan[d]

Hydrogels that support vascularization have been developed to improve the survival of skin flaps, yet establishing homogeneous angiogenic niches without compromising ease of use in a surgical setting remains a challenge. Here, pressure-driven spreadable hydrogels were developed utilizing beta-sheet rich silk nanofiber materials. These silk nanofiber-based hydrogels exhibited excellent spreading under mild pressure to form a thin coating to cover all regions of skin flaps. Deferoxamine (DFO) was loaded onto the silk nanofibers to support vascularization and these DFO-laden hydrogels were implanted under skin flaps in rats to fill the interface between the wound bed and flap using applied pressure. The thickness of the spread hydrogels was below 200 μm, minimizing physical barrier effects from the hydrogels. The distribution of the hydrogels provided homogeneous angiogenic stimulation, accelerating rapid blood vessel network formation and significantly improving the survival of the skin flaps. The hydrogels also modulated immune reactions, further facilitating regeneration of the skin flaps. Considering the homogeneous distribution at wound sites, improved vascularization, reduced barrier effects and low inflammation, these hydrogels appear to be promising candidates for use in tissue repair where a high blood supply is in demand. The pressure-driven spreading property should simplify the use of the hydrogels in a surgical setting to facilitate clinical translation.

[a] *National Engineering Laboratory for Modern Silk & Collaborative Innovation Center of Suzhou Nano Science and Technology, Soochow University, Suzhou 215123, People's Republic of China.*
[b] *Department of Plastic and Cosmetic Surgery, The Second Affiliated Hospital of Soochow University, Suzhou 215004, P. R. China.*
[c] *Department of Dermatology and Cosmetic Surgery, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou 215002, Jiangsu, China.*
[d] *Department of Biomedical Engineering, Tufts University, Medford, Massachusetts 02155, United States.*
†*Corresponding author: Qiang Lu, Tel: (+86)-0512-67061649; E-mail: lvqiang78@suda.edu.cn*
‡ *The authors contributed equally to this work.*

ARTICLE

Surgical procedure. The pressure driven hydrogel was injected between the skin flap and the basal wound surface through a needle-free syringe before the wound edge was completely closed. After the injection, the last part of the edge was sutured, then the surface of the flap was gently touched with the surgeon's hand, and the hydrogel spread homogeneously under pressure.

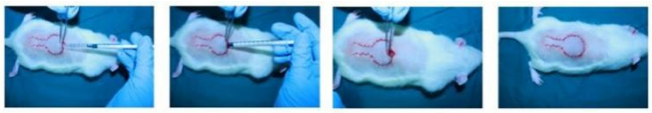

Fig. S1 The surgical procedure with injection and pressure.

Measurement of the pressure on the surgeon's finger. The result showed that the gentle pressure range is 490 g to 530 g.

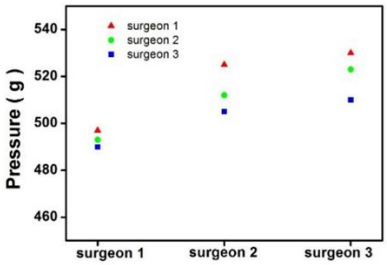

Fig. S3 The pressure that plastic surgeons applied to skin.

Improved flow and spreading capacity of BSNF hydrogels after stirring.

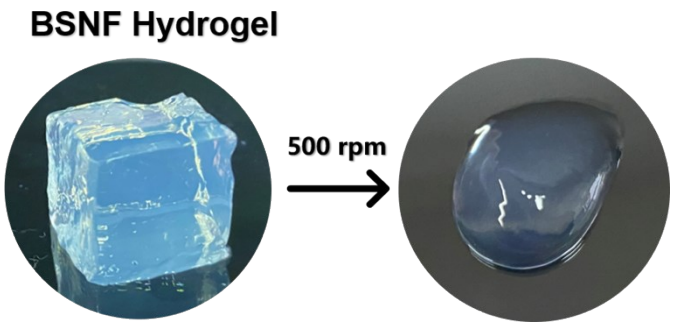

Fig. S2 Macro-morphology of 1% BSNF hydrogel before and after stirring at 500 rpm for 4h. After the stirring, the hydrogel becomes injectable and spreadable.

Swelling behaviors of the BSNF hydrogels.

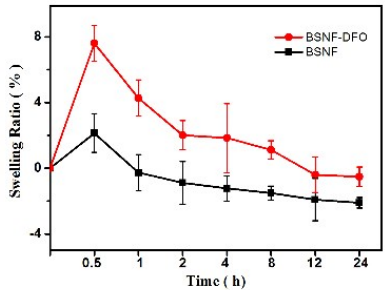

Fig. S4 Swelling behaviors of BSNF hydrogels in PBS solutions. BSNF: 1% BSNF hydrogel, BSNF-DFO: 1% BSNF hydrogel loaded with 60uM DFO.

The injectability of BSNF hydrogels. The hydrogel can be pushed out smoothly with a needle (22G), indicating that the hydrogel is injectable.

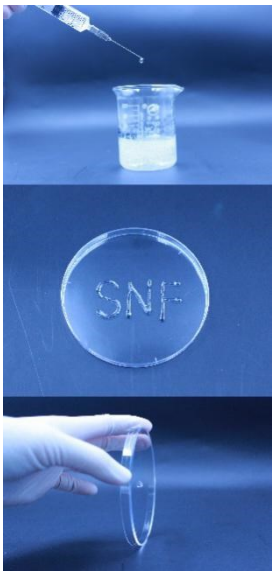

Fig. S5 The injectability of 1% BSNF hydrogels.

***In vivo* test of hydrogel volume.** Different amounts of DFO-laden BSNF hydrogels were injected under the flap.

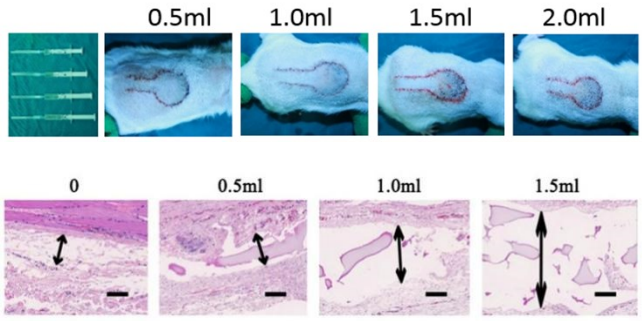

Fig. S7 Effect of hydrogel volume on survival of skin flap. Different volumes of 60uM DFO-laden BSNF hydrogels were injected under the flap and formed thin coatings. Scale bars are 100 μm. The pink masses in the interstitial space are the BSNF hydrogels with H&E staining.

Study of "Pingpong racket" skin flap model. The traditional random skin flap model has been improved previously in our group. We designed a simple and easy-to-prepare model with more non-vascular area than traditional random flap model. After the experiment and the survival area analysis, the results confirmed that the pedicle length was 3 cm, the pedicle width was 1 cm, and the round part had a diameter of 3 cm. Intraoperative observation of the pingpong-shape flap revealed the blood vessel free state of the pedicle. The pingpong racket model is suitable for the study of skin flap survival.

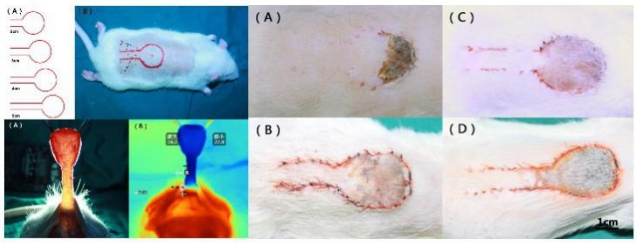

Fig. S6 The design of the skin flap model and the study of pedicle length.

***In vivo* test of DFO concentrations.** Drug concentrations were measured on random skin flaps of rats, where 0.5 mls of different DFO-laden BSNF hydrogels were administered to the rats. Skin flaps with a DFO concentration of 60uM had the best survival of the skin flaps.

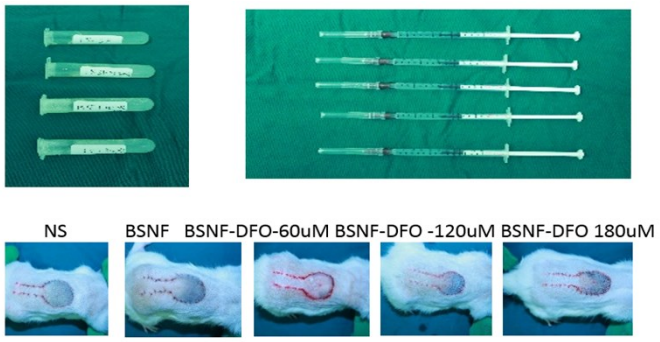

Fig. S8 The *in vivo* test of DFO concentrations.