Supporting Information

Injectable VEGF-Loaded Gelatin-Hyaluronic Acid Hydrogel to Synergistically Promote Flap Survival via Rapid Angiogenesis

Dan Zhong,[a] Lin Zhi,[b] Haitao Xiao,*[b] Xuewen Xu*[a,b]

[a] Research Laboratory of Plastic and Burns Surgery, West China Hospital, Sichuan University, Chengdu 610041, China

[b] Department of Plastic and Burn Surgery, West China Hospital, Sichuan University, Chengdu 610041, China

*Correspondence: xhaitao@163.com; xuxuewen@scu.edu.cn

1. Materials and Methods

1.1 Materials

Gelatin (type-A powder, 300 Bloom), microbial transglutaminase (mTG, 200 U/g) and vascular endothelial growth factor (VEGF, 99%) were purchased from Shanghai yuanye Bio-Technology Co. Ltd. (China). Hyaluronic acid (HA, M_W = 10 kDa) was purchased from Heowns Biochem Technologies LLC (China). Cell counting kit-8 (CCK-8) was obtained from Dojindo Laboratories (Japan). Fluorescein diacetate (FDA) and rhodamine B (RhB) was obtained from Sigma-Aldrich (USA). Crystal violet and Matrigel were purchased from Beyotime Biotechnology (China). 5-chloromethylfluorescein diacetate (CMFDA) was obtained from Yeasen Biotechnology (China).

1.2 HA and Gelatin Release

To study the release of HA and gelatin from the hydrogels, three samples of each gel were soaked in PBS (0.01 M, pH 7.4) and incubated at 37℃. At specified time points (e.g., 1, 2, 3 and 7 d), the medium was entirely withdrawn and replaced by fresh PBS. The amount of HA was determined via a uronic acid assay [1], with a standard curve established using pristine HA. And the amount of released gelatin was measured using a BCA protein assay kit. The released HA (wt%) was calculated according to the equation: released HA/total HA ×100%.

2. Supplementary Data

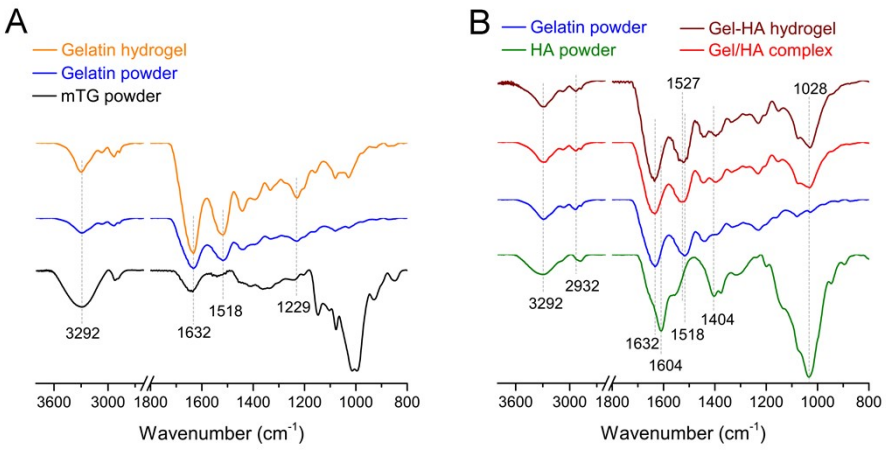

Fig S1. Photograph of the injectability of Rhodamine B-loaded Gel-HA hydrogel through needle.

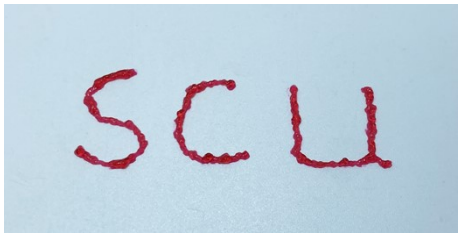

Fig S2. Photograph of the injectability of Rhodamine B-loaded Gel-HA hydrogel through needle.

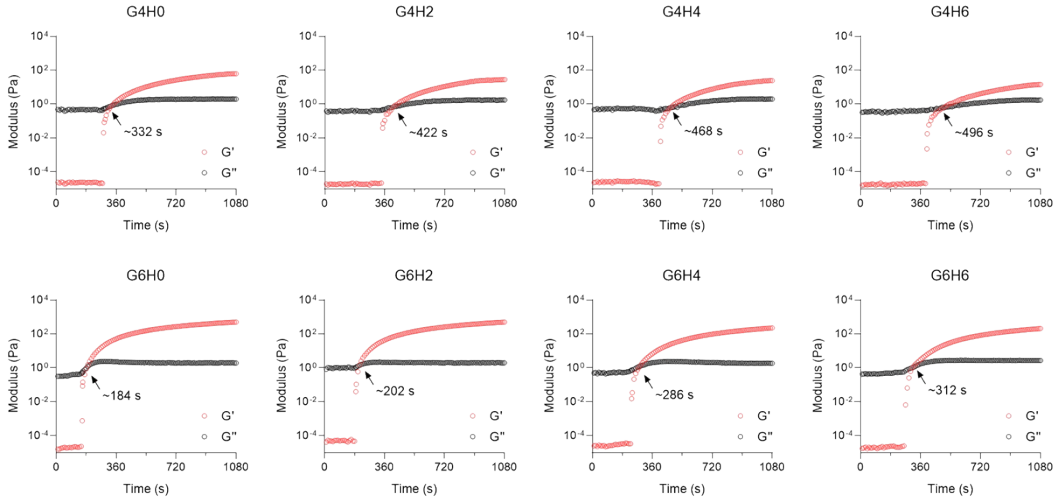

Fig. S3. Rheological properties of hydrogels. The G′ and G″ of these hydrogels at 37°C.

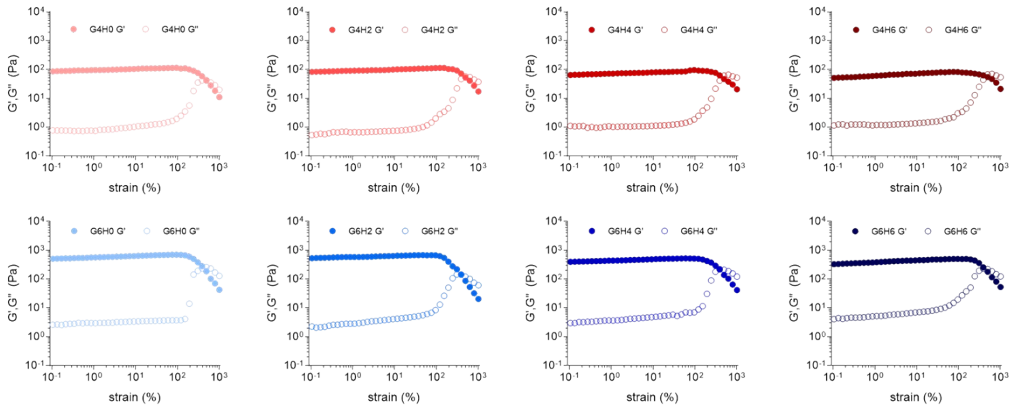

Fig. S4. The strain amplitude sweep test (γ = 0.1%-1000%) for the hydrogels at a fixed frequency of 1 Hz at 37℃.

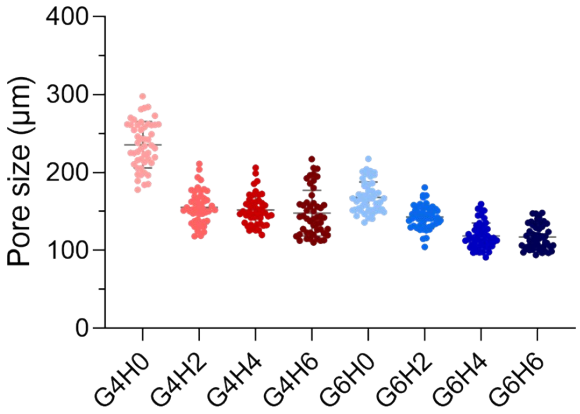

Fig. S5. The pore size of these hydrogels as calculated from SEM images.

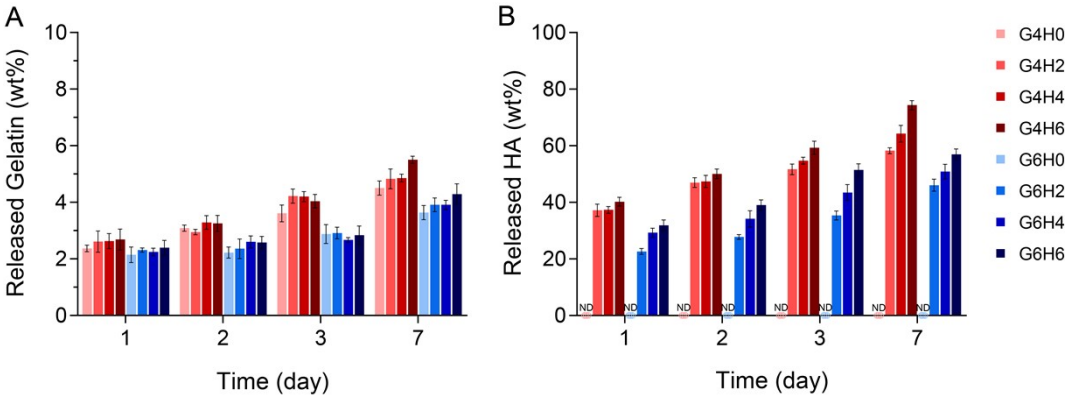

Fig. S6. Amount (wt%) of gelatin (A) and HA (B) released from the Gel-HA hydrogel in PBS at 37℃ after different incubation times. ND: not detected. **P*<0.05, #*P*<0.01.

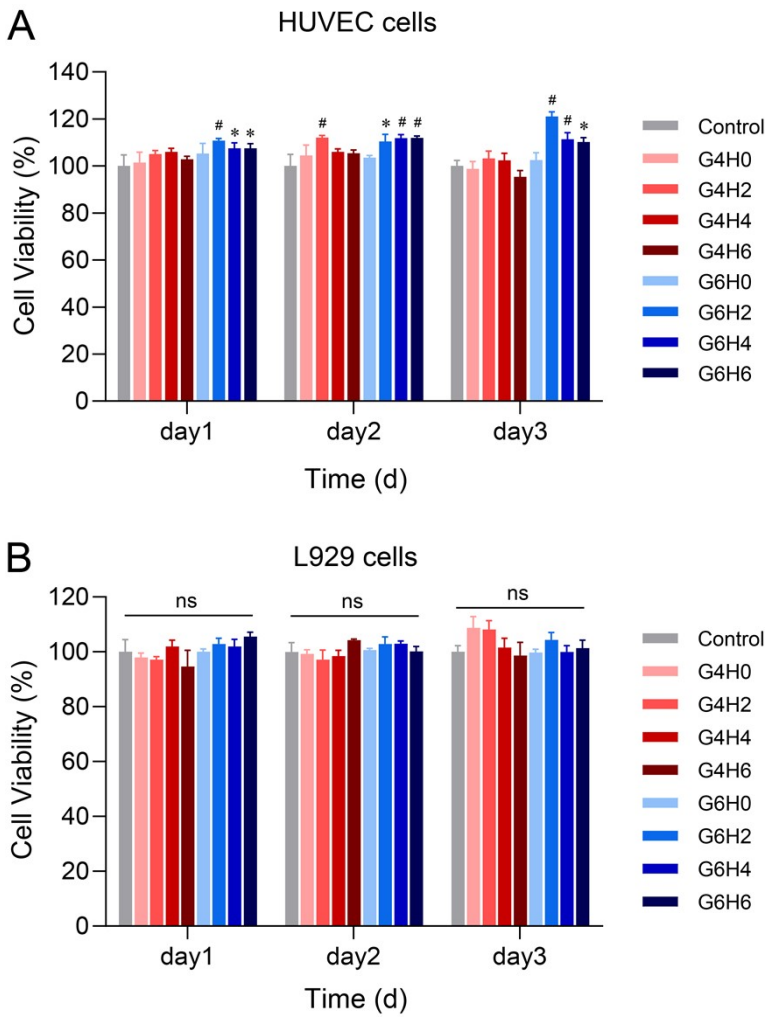

Fig. S7. Cell viability of HUVECs (A) and L929 cells (B) after treatment with Gel-HA hydrogels for 1, 2 and 3 days. ns: no significance, **P*<0.05, #*P*<0.01.

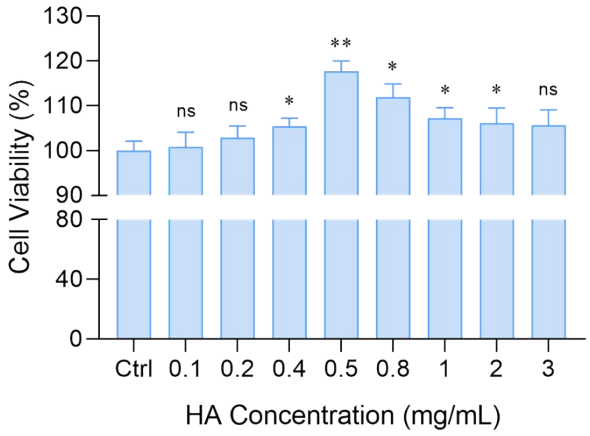

Fig. S8. Cell viability of HUVECs with the treatment of different concentrations of oligomeric HA for 48 h. ns: no significance, **P*<0.05, ***P*<0.01.

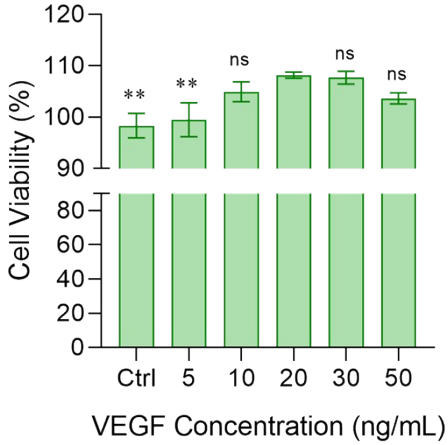

Fig. S9. Cell viability of HUVECs with the treatment of different concentrations of VEGF for 48 h. ns: no significance, $^{**}P<0.01$.

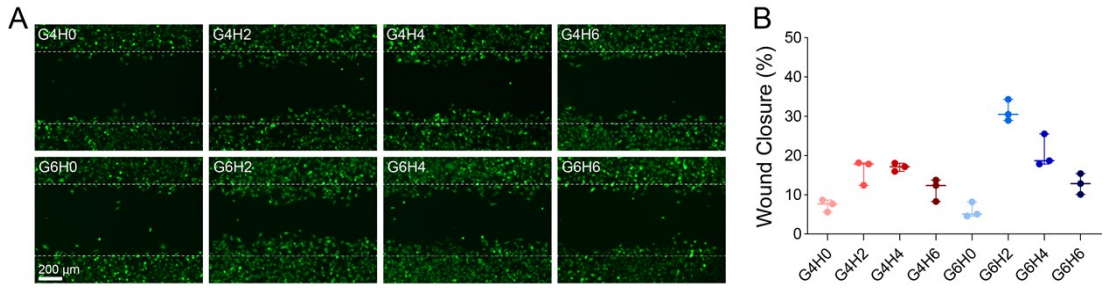

Fig. S10. (A) Scratch assay and (B) corresponding quantitative analysis evaluating the migration activity of HUVECs in the presence with Gel-HA hydrogels. The white lines indicate the scratch positions at 0 h. HUVECs were prelabeled with 5-chloromethylfluorescein diacetate (CMFDA). Scale bar: 200 μm.

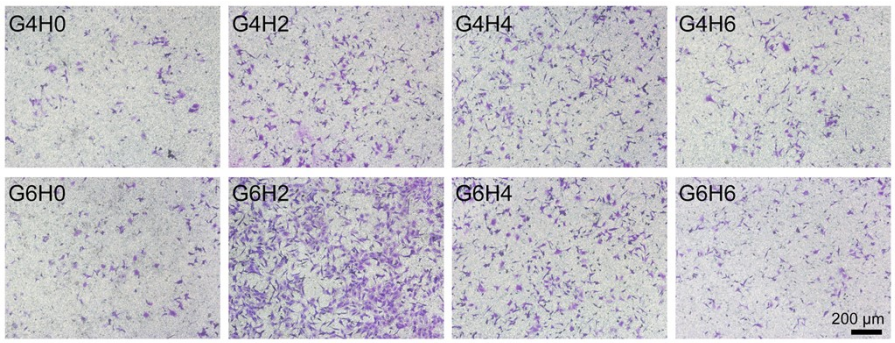

Fig. S11. Transwell assay accessing the migration activity of HUVECs in the presence with Gel-HA hydrogels. The migrated cells were stained with crystal violet. Scale bar: 200 μm.

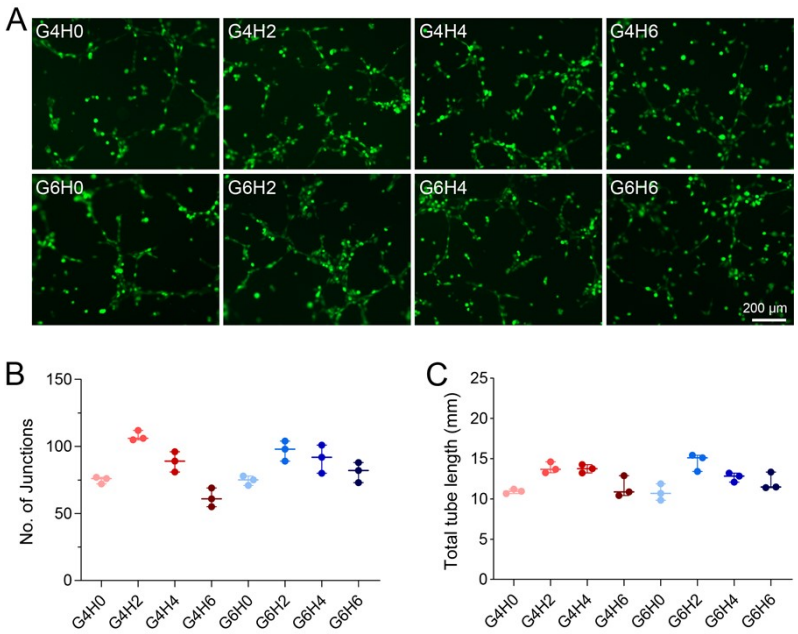

Fig. S12. (A) Optical microscope images and (B, C) corresponding quantitative analysis of the number of joint and total length evaluating the tube formation ability of HUVECs in the presence with Gel-HA hydrogels. HUVECs were prelabeled with CMFDA. Scale bar: 200 μm.

Table S1. Parameter and gelation time of Gelatin-Hyaluronic acid (Gel-HA) hydrogel samples at 37℃. GxHy represents the gelatin concentration of x and hyaluronic acid concentration of y.

Sample	Gelatin (w/v)	HA (w/v)	Gelation time (min)
G4H0	4%	-	6.62 ± 0.14
G4H2	4%	2%	7.92 ± 0.16
G4H4	4%	4%	8.66 ± 0.25
G4H6	4%	6%	9.09 ± 0.17
G6H0	6%	-	4.15 ± 0.18
G6H2	6%	2%	4.31 ± 0.17
G6H4	6%	4%	5.72 ± 0.18
G6H6	6%	6%	6.19 ± 0.15

References

1. C. Loebel, C. B. Rodell, M. H. Chen and J. A. Burdick, *Nature protocols*, 2017, **12**, 1521-1541.