Supporting Information

Release of VEGF and BMP9 from Injectable Alginate based Composite Hydrogel for Treatment of Myocardial Infarction

Yong Wu[a], Tianqi Chang[a], Weiqian Chen[a], Xiaoyu Wang[a], Jingjing Li[a], Yueqiu Chen[a], You Yu[a], Zhenya Shen*[a], Qian Yu*[b] and Yanxia Zhang*[a]

[a] Institute for Cardiovascular Science & Department of Cardiovascular Surgery of the First Affiliated Hospital, Medical College, Soochow University, Suzhou 215000, P. R. China.

[b] State and Local Joint Engineering Laboratory for Novel Functional Polymeric Materials, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou, 215123, P. R. China

Corresponding Authors

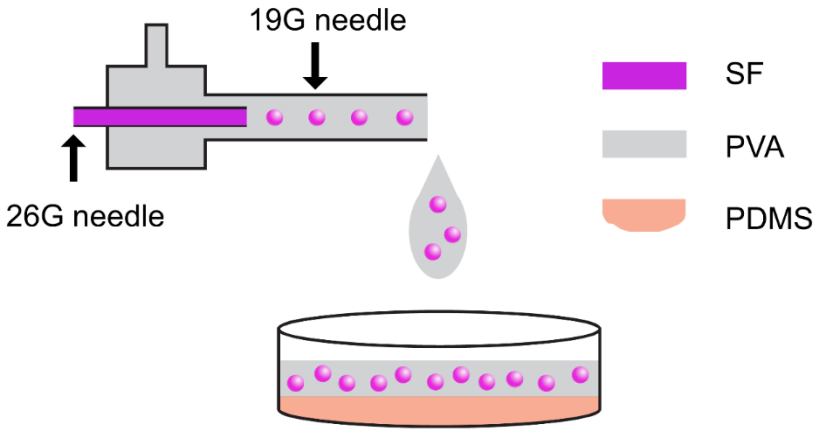
*E-mail: zhangyanxia@suda.edu.cn; yuqian@suda.edu.cn; uuzyshen@aliyun.com

S1. Experimental Section

S1.1 Preparation of SF Microspheres

SF solution was prepared as follows. Briefly, silk fibers (RudongXinsilu Co., Ltd, Jiangsu, China) were degummed in Na_2CO_3 aqueous solution, then rinsed thoroughly with deionized water and dried at 37℃. Next, 1 g silk was dissolved in 5 mL LiBr solution (9.3 M, Strem Chemicals Inc., MA, USA) at 60℃. Subsequently, the solution was dialyzed to remove residual LiBr. Finally, 10% (w/v) SF solution was obtained by concentrated dialyzed against polyethylene glycol (PEG, Mw = 20000, Biosharp, Shanghai, China) powder. SF solution was then centrifuged at 9000 rpm for 20 min and stored at 4℃ before use.

SF microspheres were prepared by a microfluidic device as indicated in **Scheme S1**. Briefly, silicone tubing attached the coaxial needle to two individual syringe pumps (Leadfluid, Baoding, China). The outer channel was a 19 G needle with an inner diameter of 0.69 mm, whereas the inner channel consisted of a 26 G needle with an inner diameter of 0.25 mm. SF solution (6% w/v) from the inner needle tube flowed out at a rate of 1 mL/h, and the poly(vinyl alcohol) (PVA, 5% w/v, Mw = 30000-70000, Sigma-Aldrich, USA) solution from the outer needle tube flowed out at a rate of 4 mL/h. The mixed solution was poured onto a polydimethylsiloxane (PDMS) surface, and then dried at room temperature. The dried blend film (1 g) was fully dissolved in deionized water (40 mL), and then centrifuged 9000 rpm three times (10 min for each time). Finally, the precipitate was freeze-dried to get SF microspheres.



Scheme S1. Schematic illustration of preparation of SF microspheres.

S1.3 Quantification of the Infract Size

5-μm-thick tissue sections were prepared and stained with Masson's trichrome (Solarbio, China) to determine the infarct size. The tissue sections were observed using optical microscopy (Nikon, SMZ745T, Japan) and the resulted images were analyzed in a blinded fashion with ImageAnalysis9 software. The infarction size, with linear approximations to account for area gaps in histology, was expressed as a percentage of the total left ventricle myocardial area myocardial area.

S1.4. Quantification of the Vessels in the Infarcted Area

5-μm-thick tissue sections were prepared and stained with immunohistochemical staining of CD31 (CST, USA) was conducted to investigate neovascularization of infarcted tissue. The vessels presented in the infarcted area were photographed using optical microscopy (Olympus, IX51, Japan). The number of vessels present per mm^2 was analyzed in a blinded fashion with ImageAnalysis9 software and was compared between the different groups.

S2. Supplementary Results

S2.1 *In Vitro* Stability of SF microspheres

SF microspheres were added to the upper chamber of a 12-well transwell plate. 2 mL of PBS was added to the lower chamber. At days 0, 1, 3, 7, 14 and 21, samples in upper chamber were collected and then freeze-dried. The morphology of SF microspheres before and after incubation were observed using scanning electron microscopy (SEM). As shown in **Figure S1**, after incubation in PBS for 21 days, there were no significant changes in the morphology and the diameter of SF microspheres, indicating that they were relatively stable in PBS.

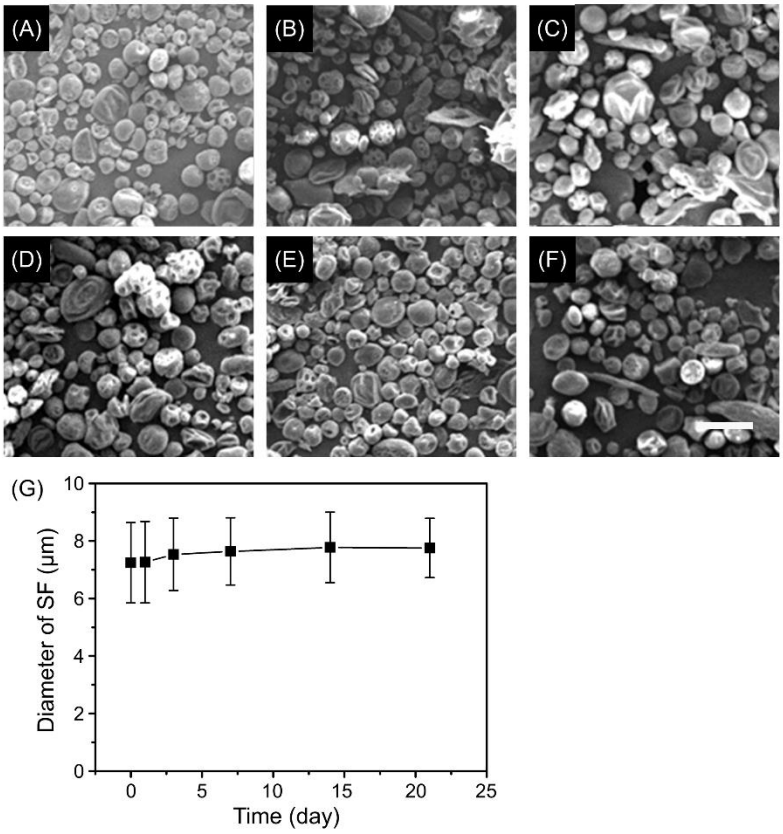


Figure S1. The morphology of SF microspheres before (A) and after (B-F) incubation in PBS for different times. (B) 1 day, (C) 3 days, (D) 7 days, (E) 14 days and (F) 21 days. Scale bar = 20 μm (G) Change in diameter of SF microspheres (n = 3).

S2.2 *In Vitro* Release of BMP9 from the Hydrogel with and without SF Microspheres

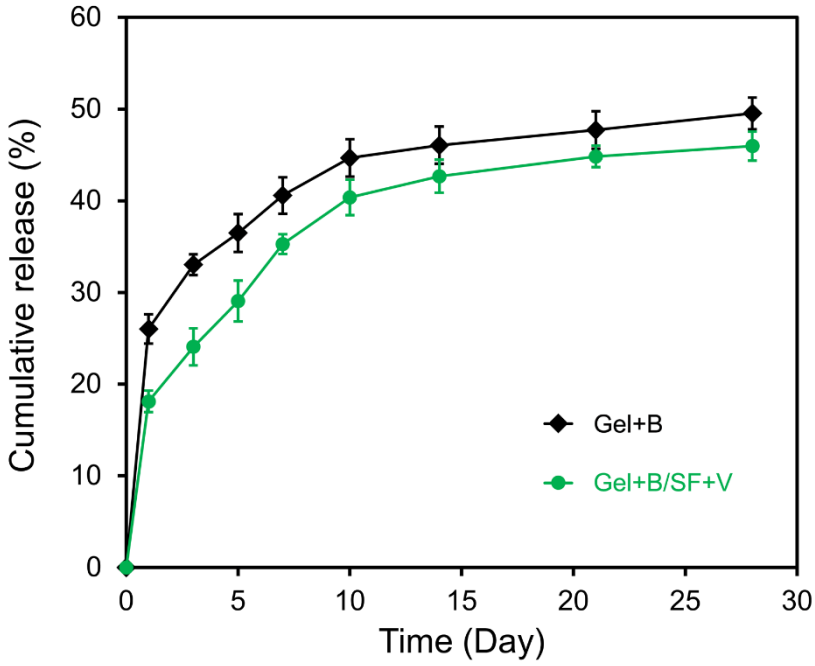


Figure 2. *In vitro* release profiles of BMP9 from Gel+B/SF+V (green curve) and Gel+B (black curve) (n = 3).

S2.3 Bioactivity of Released VEGF from the Composite Hydrogel

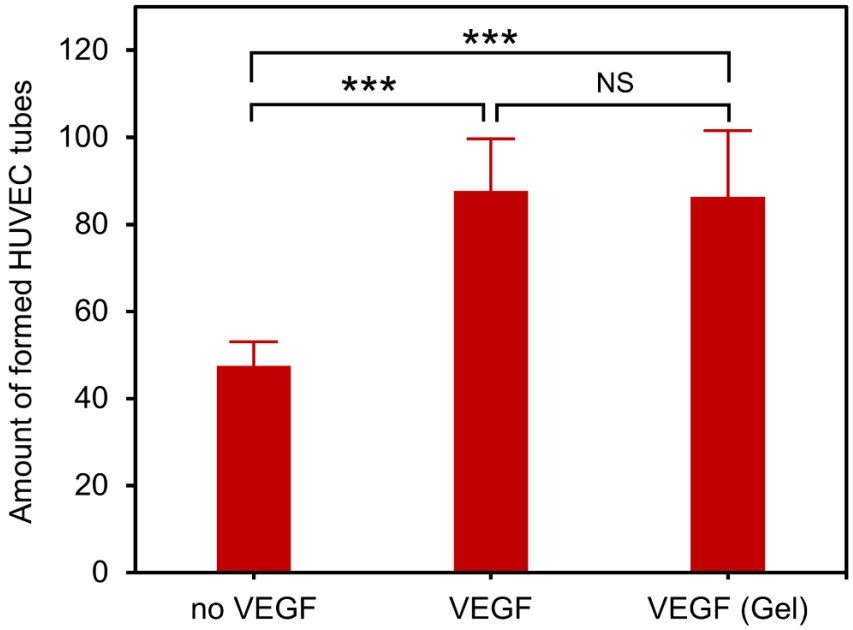


Figure S3. The amount of formed HUVEC tubes in different groups (n = 3, ***p < 0.001).