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Controlled dual delivery of low doses of BMP-2 and VEGF in a silk fibroin-nanohydroxyapatite scaffold for vascularized bone regeneration

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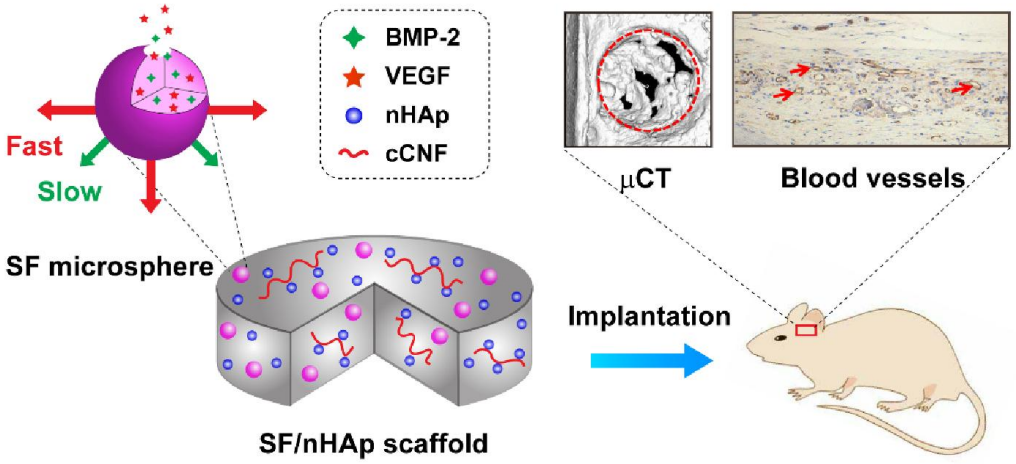

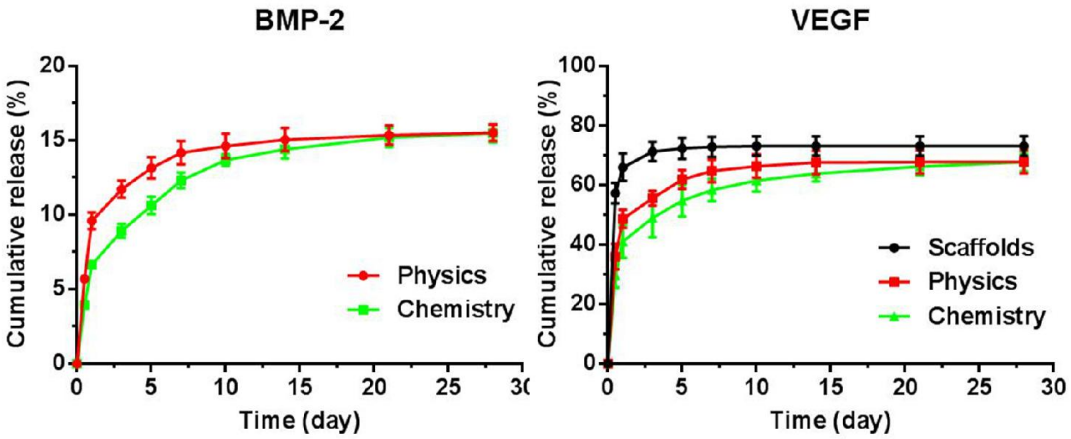

Fig. S1. In vitro release profile of the growth factors from the composite scaffolds in different ways. The growth factors were bound to SF spheres via physical and chemical (EDC/NHS) bonds. The chemically bonded factors released much more slowly compared with the physically bonded factors. The scaffolds represent that VEGF were absorbed onto the scaffold directly without using the SF microspheres. The encapsulation of VEGF into the SF microspheres prior to incorporation into an SF/nHAp scaffold was demonstrated to be an efficient strategy to deliver VEGF in a controlled release manner.

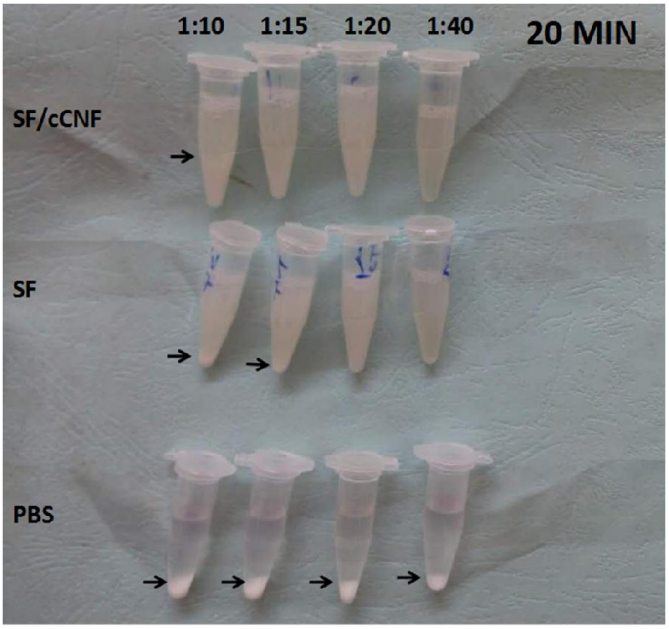

Fig. S2. The suspension stability of nHAp in different solutions of PBS, SF and SF/cCNF after 20 min. The line indicates an interface between solid and liquid phase. When the ratio of nHAp and SF increased from 1:40 to 1:10, the suspension stability decreased. The suspension of nHAp and SF/cCNF shows the best stability. nHAp:SF/cCNF = 1:15 and SF/cCNF solution were used to prepare scaffolds.

The calculation method of the amount of BMP-2 and VEGF in the scaffold:

The total amount of BMP-2 and VEGF was defined as the amount of molecules that were initially loaded into scaffold. In this work, 6 μg BMP-2 (1 μg/μL, 6 μL) and 0.4 μg (0.1 μg/μL, 4 μL) VEGF were added to 3 mg microspheres, and these microspheres were then added to 600 μL SF/nHAP solution. 30 μL of such suspension was then transferred into a mold plate for freeze-drying to get a scaffold with 5 mm in diameter and 2 mm in height. For one batch, around 20 scaffolds were prepared. Therefore, for one resulting scaffold, the amount of BMP-2 and VEGF is calculated as 300 ng and 20 ng (6 μg and 0.4 μg divided by 20), respectively.