Supporting information

**Composite microgels for imaging-monitored tracking of the delivery of vascular endothelial growth factor to ischemic muscle**

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Number of pages: 7
Number of figures: 6

**S1. Fourier transform infrared (FTIR) spectroscopy**

Figure S1 also shows the FTIR spectra of the SF samples before and after HRP/H₂O₂ mediated crosslinking. Gelatin exhibits characteristic absorption bands at 1557, 1702, and 1237 cm⁻¹, corresponding to vibrations of the C=O (amide I), N-H (amide II) bonds, N-H and C-N (amide III), respectively ¹,². In the IR spectrum of Gel(Ph), the band in the range of 3000-3300 cm⁻¹ was ascribed to the OH stretch of phenols. A band representing C-O stretch of phenols at 1243 cm⁻¹ indicated the conjugation of the aromatic phenol group with -COOH group ³,⁴. In the IR spectrum of crosslinked Gel(Ph), the peak at 2928 cm⁻¹ was indicative of diphenol groups as a product of polyphenols linked to the aromatic rings by C–C and C–O coupling to form a polymer network ⁵.

Figure S1 also shows the FTIR spectra of the SF samples before and after HRP/H₂O₂-mediated crosslinking. For the SF solution, the peaks at 1661, 1541, and 1242 cm⁻¹ were ascribed to amide I, II, and III absorption bands, respectively. The band at 1058 cm⁻¹ was assigned to the C-C mode of amino acid side chains. The spectrum of the SF/HRP/H₂O₂ mixture exhibited a small shoulder at 1630 cm⁻¹ due to SF cross-linking with HRP/H₂O₂, which was assigned to β-sheet
conformational transition in SF. In addition, the position of the amide II band shifted from 1541 to 1530 cm⁻¹, revealing a higher β-pleated sheet crystallinity of SF upon enzymatic crosslinking. The a shift of the amide III band from 1242 to 1235 cm⁻¹ suggested changes in the secondary structure of SF. The spectrum of the enzymatically crosslinked hydrogel GS showed that most of IR absorption peaks of Gel(Ph) and SF overlapped, because Gel(Ph) and SF are both protein. The peak located at 1626 cm⁻¹ was attributed to β-sheet conformation of SF during enzymatic crosslinking of GS hydrogel.

**Figure S1.** (A) FTIR spectra of Gel, Gel(Ph), G’, SF, SF- H₂O₂/HRP and GS4”.

**S2. Gelation time of Gel(Ph) and Gel(Ph)-SF solutions**

**Figure S2.** Gelation time of Gel(Ph) and Gel(Ph)-SF hydrogel as a function of HRP/H₂O₂ and SF concentration. (a) Effect of H₂O₂ and HRP concentration on gelation time at Gel(Ph) concentration of 8 wt%. (b) Effect of SF concentration on gelation time of Gel(Ph)-SF hydrogel.
at \( \text{H}_2\text{O}_2 \) concentration of 1 mM. The experimental points represent the mean (\( n = 3 \)) and standard deviation.

**S3. Simulation and design of the microfluidic device**

Droplet generation was simulated with the “Two-phase Flow, Level Set” module in COMSOL Multiphysics 5.5, and the “Time-Dependent with Phase Initialization study” was used for the computations. Figure S3 shows the design and dimensions of the microfluidic (MF) droplet generator. The width of the microchannels carrying aqueous solutions and the continuous phase were 50 µm and the orifice width was 50 µm. The width of the main channel downstream of the orifice was 200 µm. The height of the channels in the MF device was 150 µm.

Fluid flow within MF channels was regarded as laminar flow. Besides the no-slip boundary condition on walls, the surface inside the microchannel was considered as a wet wall with a constant contact angle of \( \pi/4 \). The flow rates and pressure were chosen as the inlet and outlet boundary conditions, respectively. The sensitivity of the geometry to the grid size and type of the element was examined, in order to ensure the accuracy of the modeling. Free triangular elements with a maximum and minimum size of 1.54 and 0.00461 µm are employed for each simulation, respectively. Also, by increasing the order of elements instead of multiplying the number of them, the results were obtained with reduced computing time. All the simulations were performed on a high-performance computer at the University of Tehran (Intel Xeon Processor 2620, and 32 GB RAM).

![Figure S3. Schematic of the MF droplet generator used for the preparation of composite microgels.](image)

**S4. Microfluidic generation of microgel droplets**

We examined the effect of the oil flow rate on the dimensions of droplets and the corresponding microgels. Figure S4 shows the as-prepared microgels and their size distribution. The diameter of droplet reduced with increasing flow rate of the oil phase from 30 to 70 µL/min.
at a constant aqueous phase flow rate \( Q_{\text{Gel(Ph)}} = Q_{\text{SF}} = Q_{\text{crosslinker}} = 3 \, \mu\text{L/min} \). The diameters of the droplets were controllable and reproducible.

**Figure S4.** Optical microscopy image of the generation of droplets and size distribution of composite microgels at the oil flow rate of (A) \( Q_{\text{oil}} = 30 \), (B) 40, (C) 60, and 70 \( \mu\text{L/min} \). Dark-red arrows show supply of the dispersed phase: (Gel(Ph) mixed with HRP; SF mixed with VEGF-CDs; and a cross linker (\( \text{H}_2\text{O}_2 \))). Dark-blue arrows show the supply of the continuous phase (mineral oil mixed with 3\% (w/w) Span 80 surfactant). Scale bars indicate 400 \( \mu\text{m} \).

**S5. Flow cytometry analysis of released VEGF from microgels on endothelial cell lineage markers CD31 and VEGFR-2**

The content of endothelial progenitor markers was characterized with flow cytometry. Figure S5 shows representative flow cytometry contour plots. Corresponding isotypic controls
were used to detect unspecific fluorescence. A shift in fluorescence intensity between the isotype control and the cells stained with antibodies indicated that a high fraction of cells were CD31- and VEGFR-2-positive after their treatment with medium containing VEGF-CDs, following their release from G, GS1, GS2, and GS4 microgels on Day 14. The expression of endothelial-specific cell surface markers CD31 and VEGFR-2 was 58, 61, 60, and 62% for G, GS1, GS2, and GS4 microgels, respectively. These results confirmed that VEGF released from the microgel interior retained its bioactivity and stimulated an angiogenic response in HUVECs.

**Figure S5.** Representative flow cytometry contour plots of CD31, VEGFR-2 under released VEGF from the G, GS1, GS2, and GS4 microgels on HUVECs after 14 days.
S6. Surgical procedure of hind limb ischemic

Figure S6 illustrates the surgical procedure for the preparation of hind limb ischemic in mice. First, intraperitoneal xylazine: ketamine was injected and the hair on right hind limbs of each mice was removed with an electric shaver. The skin on the top of thighs was incised to expose the arteries, veins, and nerves. The right femoral artery was excised with surgical silk suture and the hair from the body was removed by shaving and chemical depilation before mice were scanned to avoid autofluorescence signals. The left leg hindlimb served as a control.

Figure S6. Procedure for preparing the hind limb ischemic in mice. (A) Intraperitoneal injection of xylazine: ketamine. (B) Removal of the hair on right hind limbs of each mice with an electric shaver. (C) Incision of the skin on the top of thighs to expose the arteries, veins, and nerves. (D) Incision of the right femoral artery with surgical silk suture. (E) and (F) Removed by shaving and chemical depilation of hair from the body by shaving and chemical depilation, respectively, before mice.

References
