Supplementary Information

Engineered $Fe(OH)_3$ nanoparticle-coated and rhBMP-2-releasing PLGA microsphere scaffolds for promoting bone regeneration by facilitating cell homing and osteogenic differentiation

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1. Materials and methods

1.1 RhBMP-2 release

In vitro rhBMP-2 release profiles of rhBMP-2 absorbed on scaffolds (PLGAs and FePLGAs) as well as BMP-2/pDA/PLGAs and BMP-2/pDA/FePLGAs were investigated. Briefly, rhBMP-2-loaded scaffolds were placed in test tubes and immersed in 1 mL of PBS (pH = 7.4) at 37 ^{o}C under shaking at 90 rpm. At each time point, the immersed solutions were collected, and 1 mL of fresh PBS was added to replace the volume. The released rhBMP-2 was quantitatively analyzed using a Human BMP-2 ELISA kit.

2. Results and discussion

2.1 RhBMP-2 release

The release of rhBMP-2 (Figure S5) showed that the scaffolds without a polydopamine coating had a low efficiency of rhBMP-2 absorption. BMP-2/PLGAs exhibited a burst release of rhBMP-2 on the first day (27.4%) followed by a slow release of another 12.1% over the next six days. In the release curve of BMP-2/pDA/PLGAs, an initial burst release of approximately 45.8% rhBMP-2 occurred within 3 days, and a 61.7% release was achieved in two weeks. This result indicated that pDA/PLGAs had a high efficiency for immobilizing rhBMP-2, and a prolonged release behavior could be achieved due to the polydopamine coating. In the immersed solutions of BMP-2/FePLGAs and BMP-2/pDA/FePLGAs, rhBMP-2 could not be detected. However, the results for the ALP activity of mBMSCs *in vitro* and bone formation *in vivo* exhibited that

rhBMP-2 could retain its activity and induce osteogenesis when the scaffolds were immersed into complete medium or implanted *in vivo*. Thus, it was concluded that the rhBMP-2 released in PBS combined with the Fe^{3+} released from FePLGAs and pDA/FePLGAs, which led to a loss in rhBMP-2 efficacy. However, more protein was present that could combine with Fe^{3+} in the complete medium and at the femur defect site. RhBMP-2 released from BMP-2/pDA/FePLGAs maintained its effect in the complete medium of mBMSCs and *in vivo*.

Figure S1. Digital camera photo of PLGA microspheres (left) and FePLGA microspheres (right).

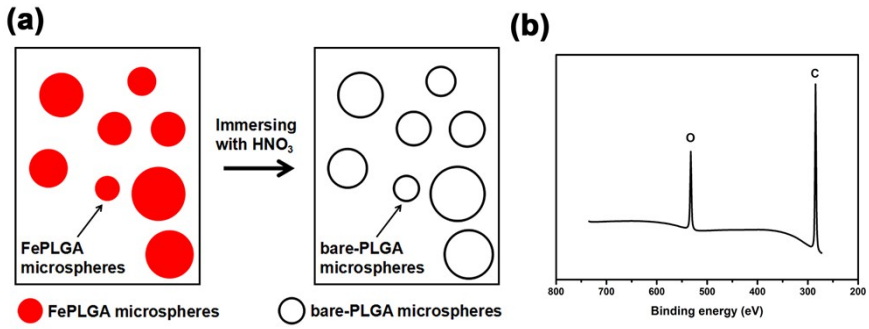

Figure S2. (a) Schematic illustration of the preparation of bare-PLGA microspheres. (b) XPS spectrum of the bare-PLGA microspheres surface.

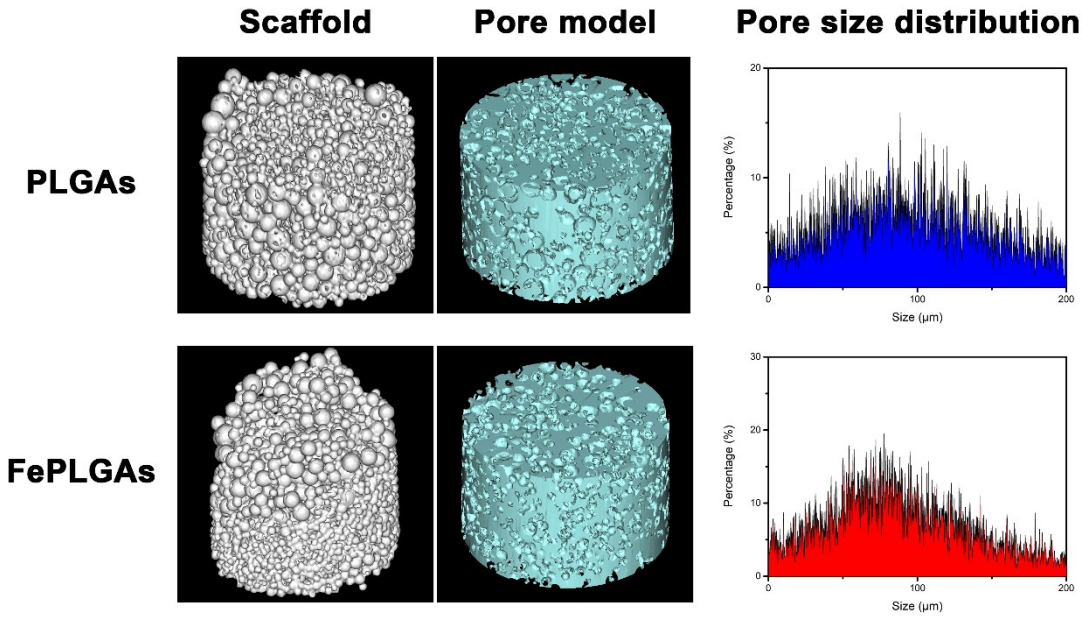

Figure S3. The structure, pore model and pore size distribution of PLGAs and FePLGAs.

Table S1. The pore property of PLGAs and FePLGAs

	PLGAs	FePLGAs
Porosity value	59.37 %	53.42 %
Pore interconnectivity value	98.51 %	99.46 %
Average pore diameter	117.29 μm	99.86 μm

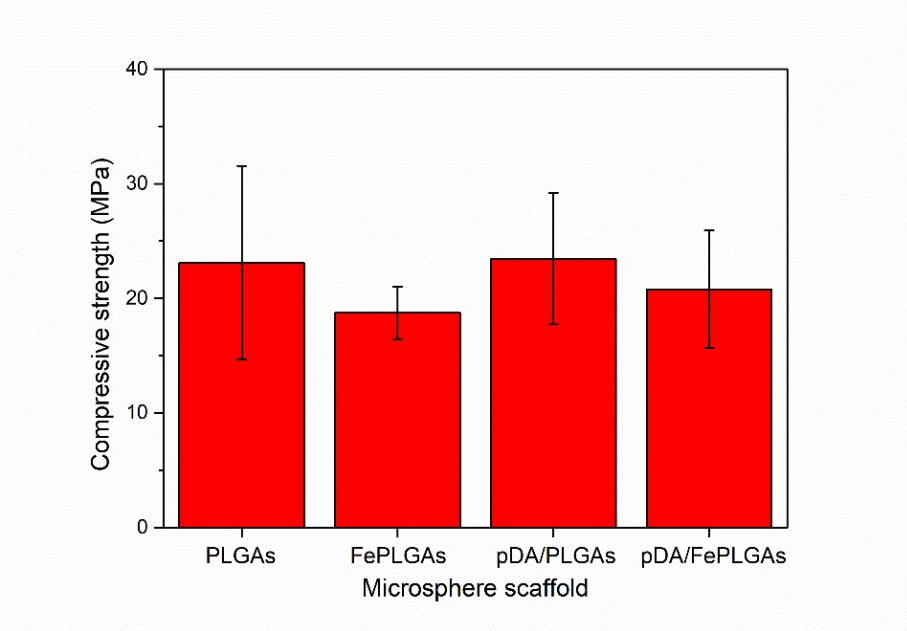

Figure S4. The compressive strength of microsphere scaffolds.

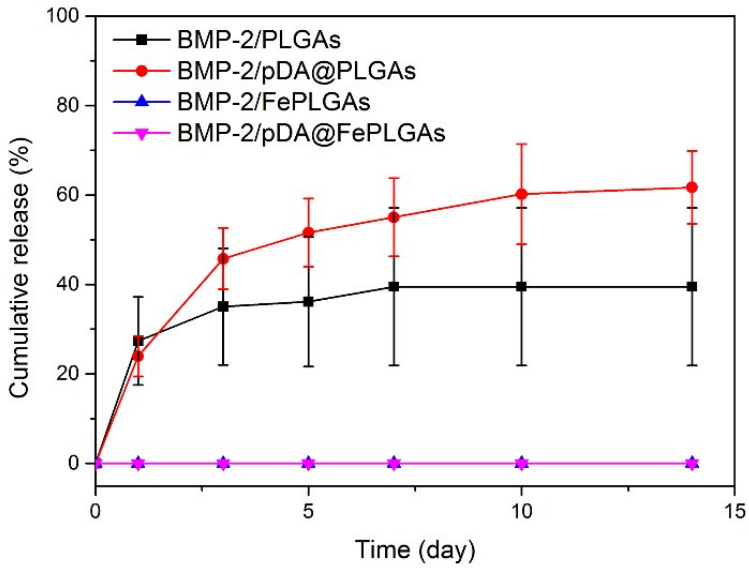

Figure S5. Kinetics of rhBMP-2 release from the scaffolds at different time periods.

Table S2. Oligonucleotide primer sequences utilized for RT-PCR

Target DNA	Primer sequence (5'-3')
GAPDH	F: GTTCCTACCCCCAATGTGTCCC; R: TAGCCCAAGATGCCCTTCAGT
ALP	F: TGCCTACTTGTGTGGCGTGAA; R: TCACCCGAGTGGTAGTCACAATG
Collagen-I	F: ATGCCGCGACCTCAAGATG; R: TGAGGCACAGACGGCTGAGTA
Runx2	F: CACTGGCGGTGCAACAAGA; R: TTTCATAACAGCGGAGGCATTTC