Support Information

In situ mineralized PLGA/zwitterionic hydrogel composites scaffolds enable high-efficiency rhBMP-2 release for critical-sized bone healing

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1. Preparation of m-PLGA/PSBMA scaffolds.

1.1. Optimization of the weight ratio of PSBMA hydrogel in mineralized PLGA/PSBMA scaffolds.

The preparation method of PLGA/PSBMA scaffolds with various weight ratio of PSBMA hydrogel was published in our previous study [1]. In this experiment, the weight ratio of PSBMA hydrogel was set as 10 wt%, 20 wt%, 50 wt% and 60 wt%. All PLGA/PSBMA scaffolds were immersed in sterile ultrapure water (pH ~ 8) for 48 h to remove the remnants and stored at -20 ℃ after freeze drying. Subsequently, the mineralized solution containing with 30 wt% of calcium chloride was prepared and 200 μL of mineralized solution was dropped on the surface of PLGA/PSBMA scaffolds with various weight ratio of PSBMA hydrogel for 30 min. Then the Ca/P-rich PLGA/PSBMA scaffolds were immersed in ammonium hydroxide (15%, v/v) for 3 h to precipitate calcium phosphate [2]. The mineralized PLGA/PSBMA scaffolds were immersed in sterile ultrapure water (pH ~ 8) for 48 h to remove the remnants and stored at -20 ℃ after freeze drying. Morphology of surface and cross-section of m-PLGA/PSBMA scaffolds were collected *via* camera.

1.2. Optimization of the volume of mineralized solution.

The PLGA/PSBMA scaffolds with 50 wt% of PSBMA hydrogel and the mineralized solution with Ca^{2+} (3.37 mol/L) were prepared. Then 50 μL, 100 μL or 200 μL of mineralized solution was dropped on the surface of PLGA/PSBMA scaffolds for 30 min. Then the Ca/P-rich PLGA/PSBMA scaffolds were immersed in ammonium hydroxide (15%, v/v) for 3 h to precipitate calcium phosphate. The purification method was referred to the above protocol. The SEM images of surface and cross-section of m-PLGA/PSBMA were measured via SEM.

1.3. Optimization of the concentration of mineralized solution.

The PLGA/PSBMA scaffolds with 50 wt% of PSBMA hydrogel were prepared according aforementioned protocol. The molar concentration of Ca^{2+} in mineralized solution was set at 1.69, 3.37 and 4.21 mol/L, and 200 μL of mineralized solution was dropped on the surface of PLGA/PSBMA scaffolds for 30 min. Then the Ca/P-rich PLGA/PSBMA scaffolds were immersed in ammonium hydroxide (15%, v/v) for 3 h to precipitate calcium phosphate. The purification method was referred to the above protocol. Morphology of surface and cross-section of mineralized PLGA/PSBMA scaffolds were collected *via* camera..

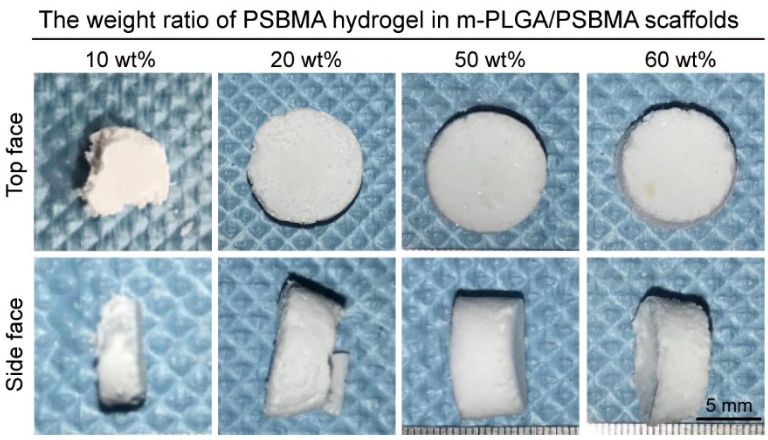

Fig. S1. Morphology of mineralized PLGA/PSBMA scaffolds containing with various weight ratio of PSBMA hydrogel. The mineralized PLGA/PSBMA scaffolds were mineralized in 200 μL of mineralized solution with Ca^{2+} (3.37 mol/L).

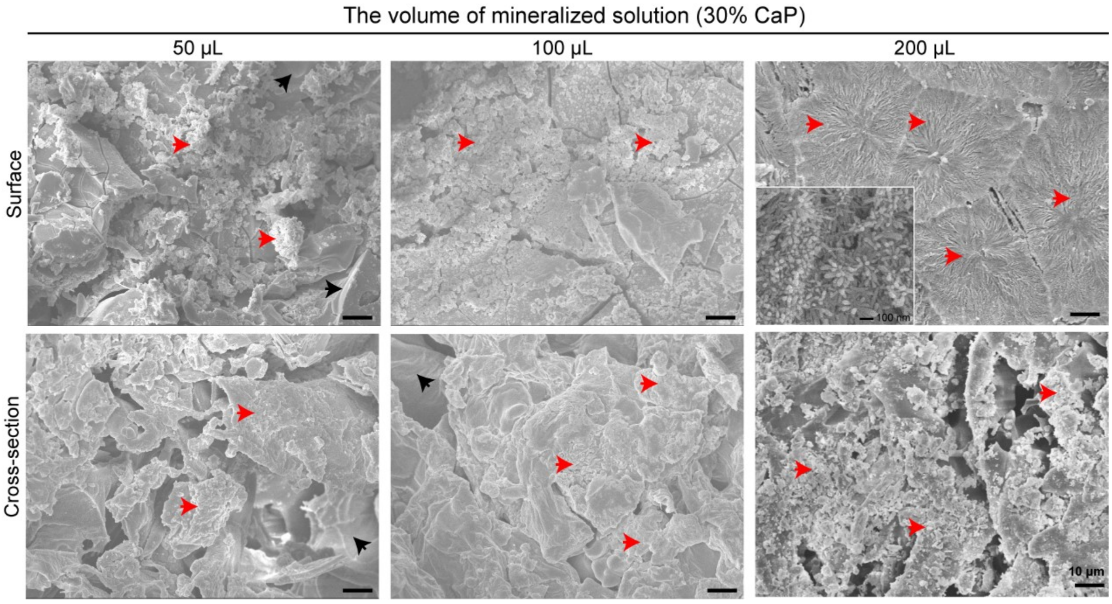

Fig. S2. SEM images of the surface and cross-section of mineralized PLGA/PSBMA. The weight ratio of PSBMA hydrogel was 50 wt%. The molar concentration of Ca^{2+} was served as 3.37 mol/L, and the volume of mineralized solution was set at 50 μL, 100 μL or 200 μL, respectively.

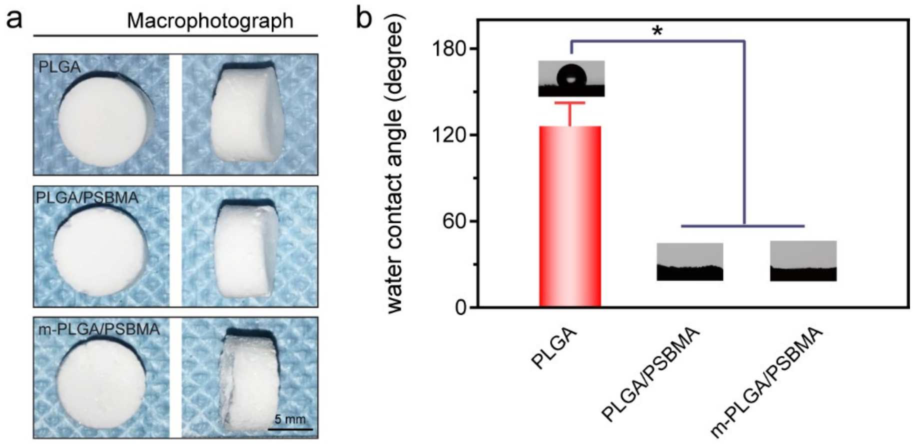

Fig. S3. (a) The representative images of PLGA, PLGA/PSBMA and m-PLGA/PSBMA scaffolds. (b) Images and quantitative analysis of water contact angle of PLGA, PLGA/PSBMA and m-PLGA/PSBMA scaffolds. All data were presented as the mean ± standard error (SEM) and all differences are significant (*$P < 0.05$, two-way ANOVA) unless denoted as NS (not significant).

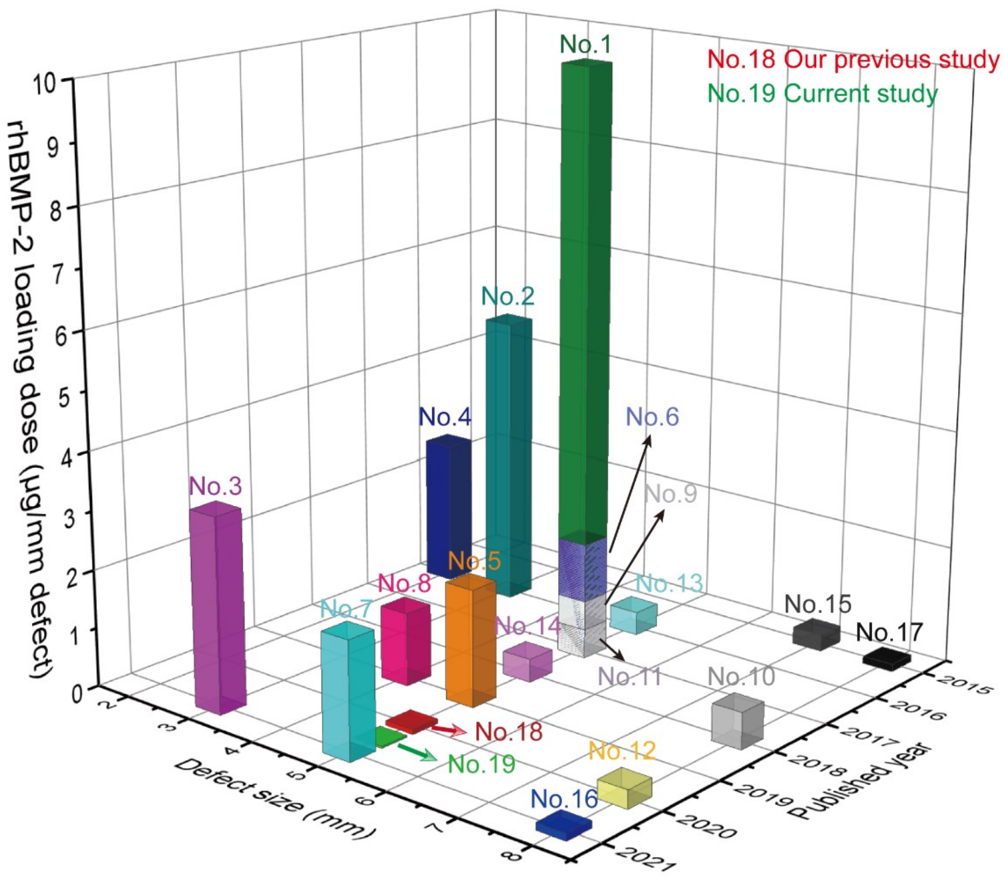

Fig. S4. Summary of rhBMP-2 loading doses on various biodegradable scaffolds for effective healing of critical-sized bone defects in recent five years. The co-delivery of rhBMP-2 with other growth factors, peptides or cells is not included.

Table. S1 Summary of rhBMP-2 loading doses on various biodegradable scaffolds for effective healing of critical-sized bone defects in recent five years. The co-delivery of rhBMP-2 with other growth factors, peptides or cells is not included.

No. in Fig. 8	Defected size (mm)	Year	rhBMP-2 loading dose (μg/mm defect)	Ref.
1	5	2017	10	[3]
2	3	2016	5	[4]
3	3	2021	3.33	[5]
4	2	2016	2.5	[6]
5	5	2019	2	[7]
6	5	2017	2	[8]
7	5	2021	2	[9]
8	4	2019	1.25	[10]
9	5	2017	1	[11]
10	8	2018	0.625	[12]
11	5	2017	0.5	[13]
12	8	2020	0.31	[14]
13	5	2016	0.4	[15]
14	5	2018	0.4	[16]
15	7	2015	0.286	[17]
16	8	2021	0.125	[18]
17	8	2015	0.125	[19]
18	5	2020	0.08	[1]
19	5	2020	0.03	*

* The current study.

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