Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Incorporating the BMP-2 Peptide in Genetically-engineered Biomaterials Accelerates Osteogenic Differentiation

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Circular Dichroism (CD)

Protein solutions (0.2 mg/mL in Milli-Q water) were scanned on a Jasco-815 Circular Dichroism spectrometer at room temperature using a 0.1 cm path length. The spectrum is an average of three scans.

Surface Quantification

The density of proteins adsorbed on TCP was quantified by using the QuantiPro BCA assay kit. Briefly, 50 μL of PBS was added to the protein surface and mixed with an equal amount of the reagent. After incubation for 1 h at 60 °C, the absorbance at 562 nm was measured. A standard curve was created using the individual proteins in PBS. Five or six replicates per group were examined.

Cell Culture

To investigate the effect of proteins that may be desorbed from the plate, cells were seeded on TCP and cultured in pre-conditioned medium. First, RZ-BMP proteins were adsorbed on TCP as described in the main text. Next, medium (low-glucose Dulbecco's modified Eagle's medium (DMEM) containing 100 U/mL penicillin and 100 mg/mL streptomycin (Invitrogen)) was incubated on either TCP or RZ-BMP surfaces for three days at 37 °C and 5% CO_2. The medium was then supplemented with 10% fetal bovine serum (Lonza), 100 nM dexamethasone, 50 μM ascorbic acid 2-phosphate, and 10 mM glycerol-2-phosphate disodium salt hydrate. The osteogenic pre-conditioned medium was added to cells grown on TCP after they reached a confluence of 70-85%. The pre-conditioned medium was replaced every three days.

Statistical Analysis

All data are represented as mean ± standard deviation. All data passed normality testing by Shapiro-Wilk test and homogeneity of variance by Levene's modified test. A one-way (Figure S1B and S4) or two-way (Figure S1A) analysis of variance (ANOVA) was performed. To determine significant differences between groups, a Tukey's *post hoc* test (Figure S1), a two-tailed t-test (Figure S3), or Dunnett's test (Figure S4) was performed. All statistical analyses were performed using Statistical Analysis Software (SAS, version 9.2), and a *p*-value less than 0.05 was considered statistically significant.

Table S1. Primer sequences for quantitative reverse transcription-polymerase chain reaction.

Gene	Accession number	Primer sequence (5' → 3')		Efficiency	Product length (bp)	Reference
Runx2	NM004348.3	F	CTTCAAGGTGGTAGCCCTCG GAGAG	97%	106	Designed
		R	AACAGCAGAGGCATTCCGG AGC			
AP	NM00478.4	F	GTACTGGCGAGACCAAGCG CAA	96%	110	Designed
		R	CACCCATCCCATCTCCCAGG AACA			
Type I col	NM000088	F	TCTGACTGGAAGAGTGGAG AGTACTGG	102%	139	[1]
		R	ACCAGTTCTTCTGGGCCACA CT			
OPN	NM001040060.1	F	GTGGGAAGGACAGTTATGA AACG	96%	134	[2]
		R	CTGACTATCAATCACATCGG AAT			
BSP	NM004967	F	GGGCAGTAGTGACTCATCCG AAG	96%	164	[3]
		R	CTCCATAGCCCAGTGTTGTA GCAG			
Type II col	NM001844.4	F	GGCAATAGCAGGTTCACGT ACA	96%	79	[4]
		R	CGATAACAGTCTTGCCCCAC TT			
GAPDH	NM002046.3	F	ACAGTCAGCCGCATCTTCTT	102%	81	[5]
		R	TTGACTCCGACCTTCACCTT			

Table S2.Amino acid analysis of RZ-BMP and RZ-scBMP proteins.

	RZ-BMP Observed mol%	RZ-scBMP Observed mol%	Calculated mol%
ASX	4.00	3.67	3.37
SER	13.36	13.95	14.61
GLX	13.68	13.71	13.48
GLY	8.43	8.40	8.43
HIS	4.48	4.81	5.06
ARG	2.32	2.10	1.69
THR	7.62	7.68	7.87
ALA	13.62	13.48	13.48
PRO	11.87	12.37	12.36
TYR	0.66	0.63	0.56
VAL	1.28	0.83	0.56
MET	2.74	3.00	3.37
LYS	5.07	5.05	5.06
ILE	1.55	1.16	1.12
LEU	3.70	3.40	3.37
PHE	5.62	5.77	5.62
Total	100.00	100.00	100.00

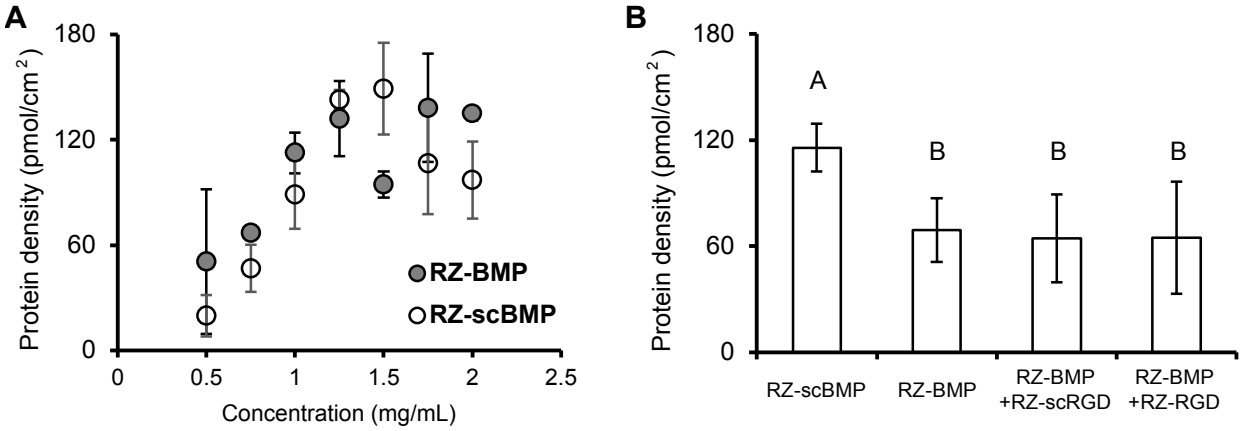

Figure S1. Adsorbed protein density. (A) Adsorption isotherms show the adsorbed protein density as a function of concentrations of the protein solutions. A two-way ANOVA was used to determine whether protein identity or concentration had a significant effect on adsorbed protein density. The protein identity did not affect adsorbed protein density, whereas the concentration did. The adsorbed protein density increased at a concentration of 0.5 mg/mL and saturated at a concentration of 1 mg/mL. Therefore, in this work, we chose a protein concentration of 1 mg/mL to prepare our surfaces. Data (n=3-5) are represented as the mean ± standard deviation. (B) For the cell differentiation studies shown in Figures 5-7, the density of the adsorbed protein surfaces was quantified. A one-way ANOVA and Tukey's *post hoc* test were performed, and different letters denote significant differences ($p < 0.05$) between groups. The RZ-BMP, RZ-BMP+RZ-scRGD, and RZ-BMP+RZ-RGD surfaces had statistically equivalent protein densities, whereas the RZ-scBMP surface had a 1.6-fold higher protein density compared to the other surfaces. Data (n=6) are represented as mean ± standard deviation.

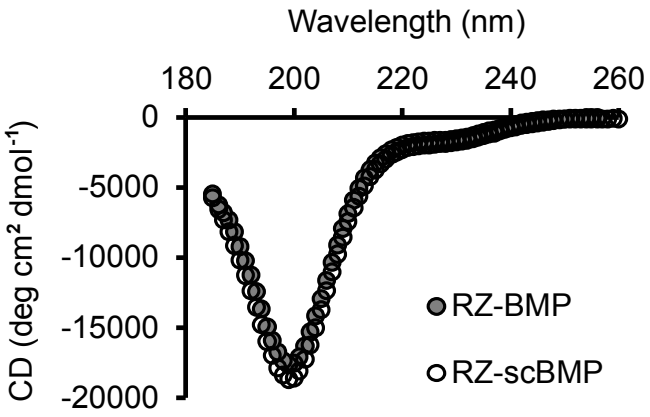

Figure S2. The secondary structure of modular proteins evaluated by CD spectroscopy. The results demonstrated that the RZ-BMP and RZ-scBMP proteins have an unordered structure. CD spectra were measured using protein solutions at 0.2 mg/mL in Milli-Q water at room temperature.

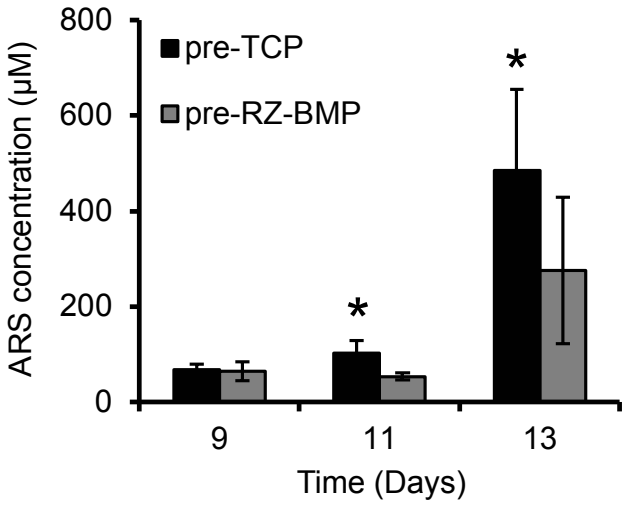

Figure S3. The effect of protein desorption on osteogenic differentiation. Human MSCs on TCP were cultured in pre-conditioned medium that had been incubated on TCP (pre-TCP) or on RZ-BMP protein surfaces (pre-RZ-BMP). Cells were stained with Alizarin red S and the amount of dye was quantified. Compared to cells cultured in pre-TCP medium, cells cultured in pre-RZ-BMP medium did not show an increase in calcium deposition. These results demonstrate that RZ-BMP protein desorption did not contribute to the results observed in Figures 4-7. The error bars indicate the standard deviation of six replicates. A two-tailed t-test was performed to determine statistical difference between two groups. * represents $p < 0.05$ compared to pre-RZ-BMP at the same time point.

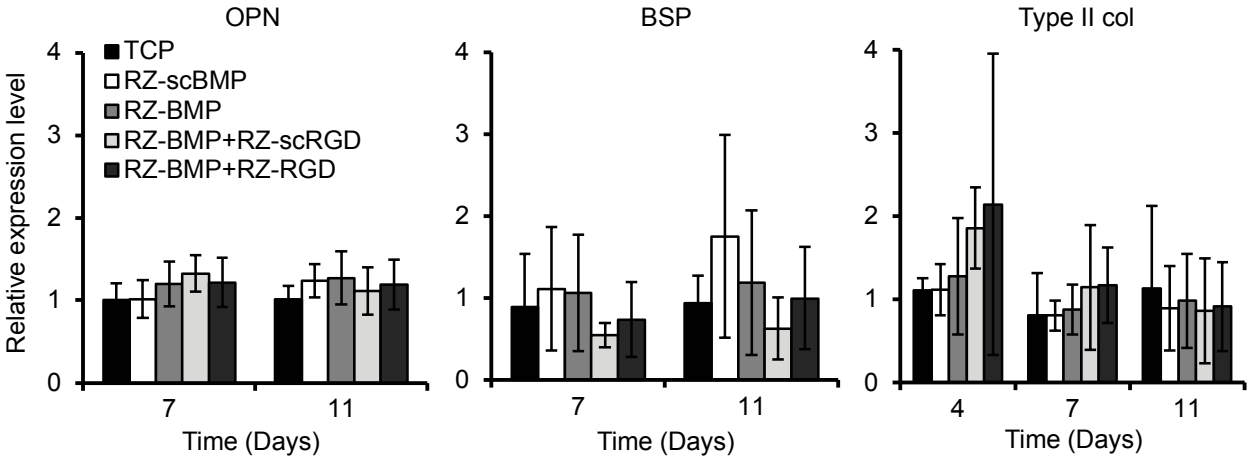

Figure S4. Relative mRNA expression levels of osteopontin (OPN), bone sialoprotein (BSP), and type II collagen (type II col) measured by qPCR. Expression levels were normalized by GAPDH (a housekeeping gene) and are reported relative to the control (TCP) at each time point. Data (n=4-6) are represented as mean ± standard deviation. Dunnett's test indicated that, at all time points, there were no statistical differences between each group and the control (TCP).

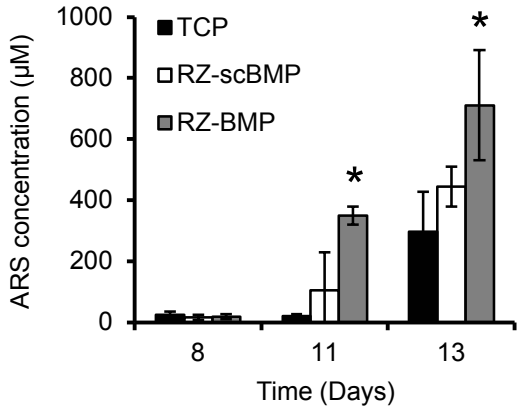

Figure S5. Accelerated calcium deposition on RZ-BMP surfaces. Cells were stained with Alizarin red S at three time points during osteogenic differentiation, and the amount of dye was quantified. At 11 and 13 days, cells grown on the RZ-BMP surfaces exhibited statistically higher calcium deposition than cells on TCP. * denotes $p < 0.05$ compared to the positive control (TCP) at that time point as determined by Dunnett's test.

References

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