Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Electronic Supplementary Information

Bioengineered mussel glue incorporated with a cell recognition motif as an osteostimulating bone adhesive for titanium implants

Yun Kee Jo, Bong-Hyuk Choi, Cong Zhou, Jin-Soo Ahn,
Sang Ho Jun, and Hyung Joon Cha**

Corresponding Author

*E-mail: (H.J.C) hjcha@postech.ac.kr, (S.H.J) junsang@korea.ac.kr

Table S1. Primers used in the study.[27]

Marker gene	Primer sequences (5'→3')		Size (bp)	Accession No.
GAPDH	Forward	AGAATTGACAAACGGGACCT	77	AY618199
	Reverse	GGAGGAGCAGAGAGCTTGAC		
Runx2	Forward	GGGGCAGTCATAACTGGGTT	115	NM_009820.5
	Reverse	GCGTGGGAACAGGTCACTTA		
BSP	Forward	CGAAGAAGCAGAAGTGGATG	111	L20232.1
	Reverse	GCTTCTTCTCCGTTGTCTCC		
ALP	Forward	GCCCTCCCGATCCTGACCAA	103	XM_006538499.2
	Reverse	GCAGAGCCTGCTTGGCCTTA		
OC	Forward	CACATGAGGGAGACAACAGG	133	L24431.1
	Reverse	GGTGATGGCCAAGACTAAGG		

[27] B. –H. Choi, H. Cheong, J. -S. Ahn, C. Zhou, J. J. Kwon, H. J. Cha and S. H. Jun, *J. Mater. Chem*., 2015, **3**, 546-555.

Table S2. Element composition of bone nodules at 21 days after differentiation of MC3T3-E1 pre-osteoblast cells on the MAP-RGD-coated Ti-mesh surface examined by EDS analysis.

Element	**C**	**N**	**O**	**Ca**	**P**	**Ti**
Composition (%)	10.27	4.13	9.81	18.79	16.04	40.96

Table S3. EDS analyses for elemental compositions of tested Ti-mesh surfaces.

Element	NC	MAP	MAP-RGD
C (%)	-	53.64	49.97
N (%)	-	21.21	23.08
O (%)	-	25.15	26.95
Ti (%)	100.00	-	-

Abbreviation: NC, bare Ti-mesh surface; MAP, MAP-coated Ti-mesh surface; MAP-RGD, MAP-RGD-coated Ti-mesh surface.

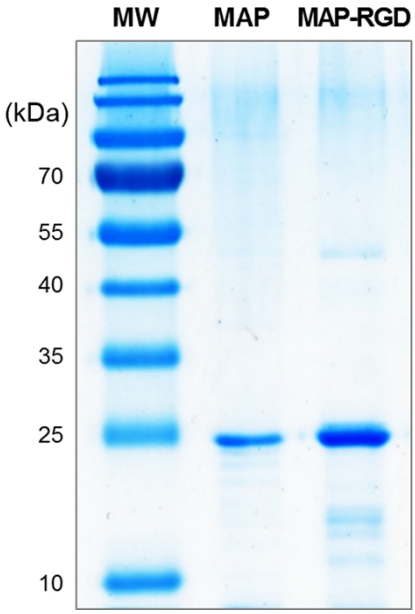

Figure S1. SDS-PAGE analysis of purified MAP and MAP-RGD.

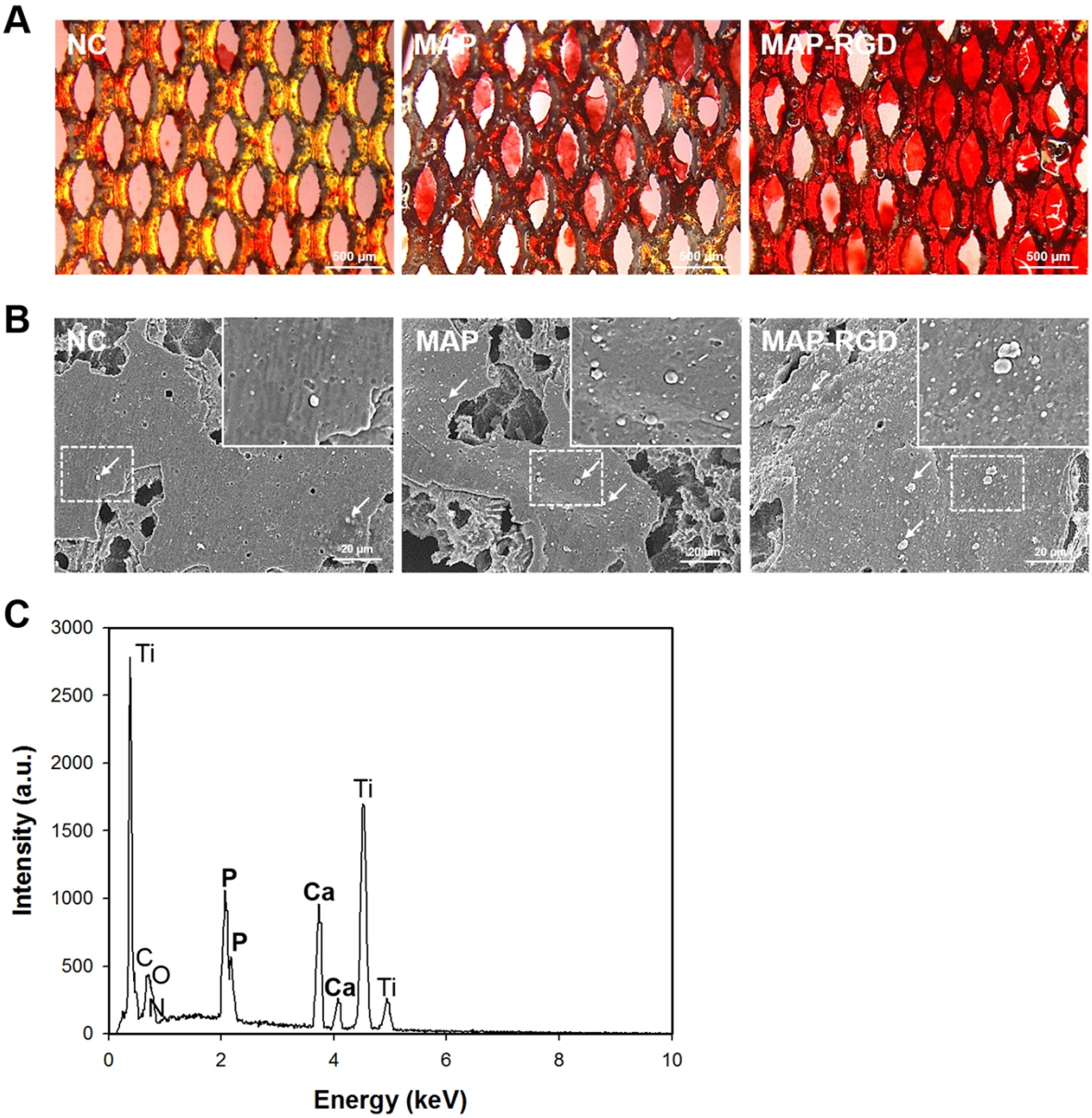

Figure S2. *In vitro* osteogenic differentiation of MC3T3-E1 pre-osteoblast cells on tested Ti-mesh surfaces. Matrix mineralization observed with (A) Alizarin red S staining and (B) FE-SEM micrographs, and (C) element composition of bone nodules examined by EDS analysis at 21 days after differentiation. Arrows in (B) indicate mineralized deposits. Abbreviations: NC, bare Ti-mesh surface; MAP, MAP-coated Ti-mesh surface; MAP-RGD, MAP-RGD-coated Ti-mesh surface.

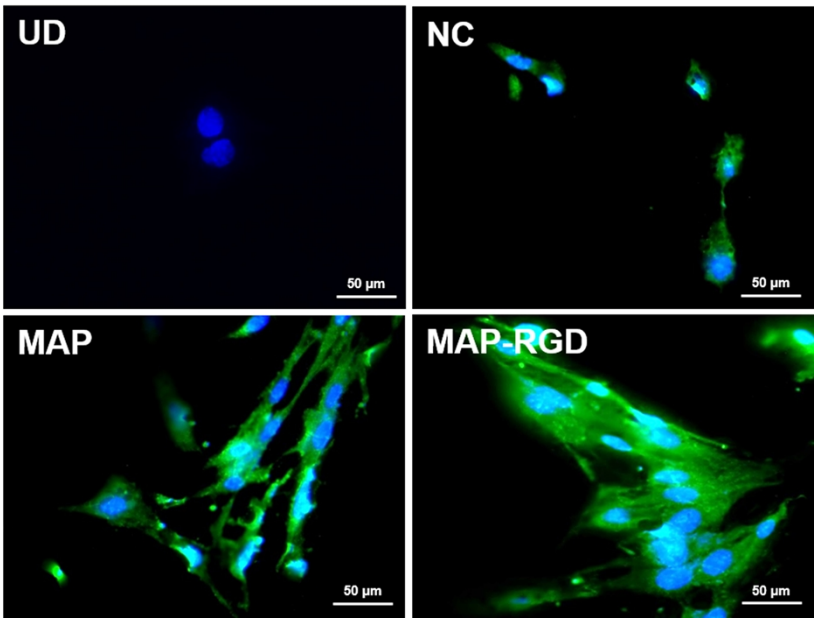

Figure S3. OC immunofluorescence staining at 15 days after differentiation. OC stained with FITC are appeared in green and nuclei stained with DAPI are blue. Abbreviations: UD, undifferentiated cells; NC, bare Ti-mesh surface; MAP, MAP-coated Ti-mesh surface; MAP-RGD, MAP-RGD-coated Ti-mesh surface.

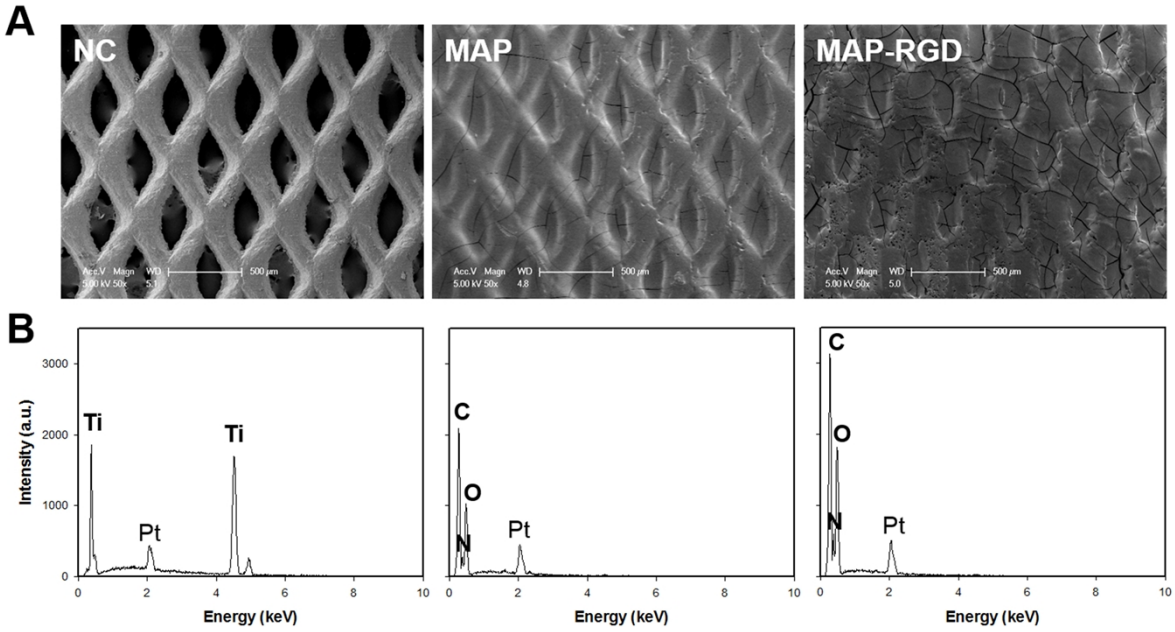

Figure S4. Surface properties of tested Ti-mesh surfaces. (A) Surface morphology examined by FE-SEM. (B) Elemental composition examined by EDS analysis. Abbreviation: NC, bare Ti-mesh surface; MAP, MAP-coated Ti-mesh surface; MAP-RGD, MAP-RGD-coated Ti-mesh surface.

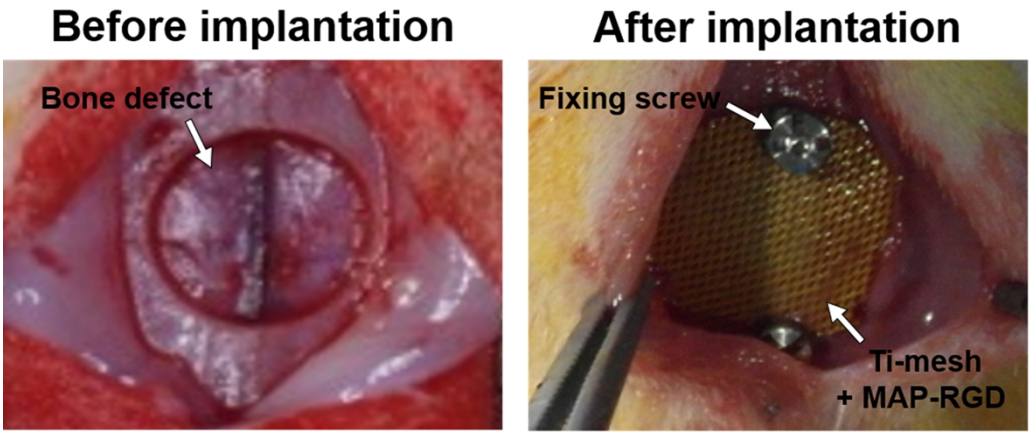

Figure S5. Creation of a calvarial defect site (left) and application of MAP-RGD-coated Ti-mesh surface with fixing screw (right) for *in vivo* animal surgery.