

Supplementary Material

Anti-Inflammatory and Osteoconductive Multi-functional Nanoparticles for

Regeneration of Inflamed Alveolar Bone defect

Hyewoo Jeong¹, Keerthana Subramanian¹, Jong-Bin Lee, Hayeon Byun, Heungsoo Shin*, Jeong-Ho Yun*

¹Hyewoo Jeong and Keerthana Subramanian contributed equally to this manuscript.

^{*}Heungsoo Shin and Jeong-Ho Yun are co-corresponding authors.

Materials

Sodium chloride (NaCl), magnesium chloride (MgCl₂), and sodium hydroxide (NaOH) were purchased from Junsei Chemicals Co. Ltd. (Tokyo, Japan). Potassium chloride (KCl), sodium phosphate dibasic (Na₂HPO₄), sodium bicarbonate (NaHCO₃), potassium persulfate (K₂S₂O₈), tannic acid (TA), collagenase, 1,10-phenanthroline, 2,2'-azino-bis(3-ethylbenzothiazoline-6sulfonic acid) diammonium salt (ABTS), iron(III) chloride hexahydrate (FeCl₃), ascorbic acid, thiazolyl blue tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), sucrose, TritonTM X-100, trizma-base, tween20 and anti-rabbit immunoglobin G (IgG) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Rapi-PlugTM S biodegradable collagen sponges were purchased from Dalim (Seoul, Korea). Phosphate-buffered saline (PBS), Dulbecco's phosphate-buffered saline, and Dulbecco's modified Eagle's medium (DMEM) were purchased from SolBio (Suwon, Korea). Fetal bovine serum (FBS) and penicillinstreptomycin (PS) were purchased from Gibco (Carlsbad, CA, USA). CEFOgroTM human MSC Growth Medium (MSC-GM) was purchased from CEFO (Seoul, Korea). LIVE/DEAD Viability/Cytotoxicity Kit and Streptavidin FITC were purchased from Invitrogen (Carlsbad, CA, USA). Lipopolysaccharide from P. gingivalis (LPS-PG) was purchased from InvivoGen (San Diego, CA, USA). Calcium chloride (CaCl₂) was purchased from DUKSAN (Gyeonggido, Korea), and hydrochloric acid (HCl) was purchased from EMD Millipore (Darmstadt, Germany). Tris (hydroxymethyl) aminomethane hydrochloride (TRIS-HCl) was purchased from IBI Scientific (Dubuque, IA, USA). The RNeasy Mini Kit was purchased from Qiagen (Valencia, CA, USA), Maxime RT Premix from Intron (Seoul, Korea), and SYBR Premix Ex Taq from TAKARA (Otsu, Shiga, Japan). The anti-mannose receptor (anti-CD206), antiinducible nitric oxide (iNOS), anti-osteopontin (OPN), and anti-runt-related transcription factor 2 (RUNX2) antibodies were purchased from Abcam (Cambridge, MA, USA). Mounting medium containing 4', 6-diamidino-2-phenylindole (DAPI) was bought from

Vectashield® (Burlingame, CA, USA), and 4% paraformaldehyde phosphate buffer solution was obtained from Wako Pure Chemical (Osaka, Japan).

Table S1. Murine inflammatory primer sequences used for RT-PCR

Primer Name	Primer Sequence (5' → 3')
GAPDH Fw	AAC TTT GGC ATT GTG GAA GG
GAPDH Rv	ACA CAT TGG GGG TAG GAA CA
iNOS Fw	CCC CGC TAC TAC TCC ATC AG
iNOS Rv	CCA CTG ACA CTT CGC ACA AA
MIP-1α Fw	CAA CCA AGT CTT CTC AGC GC
MIP-1α Rv	CTT TGG AGT CAG CGC AGA TC
IL-1β Fw	GAC CTT CCA GGA TGA GGA CA
IL-1β Rv	AGC TCA TAT GGG TCC GAC AG

Table S2. Human osteogenic primer sequences used for RT-PCR

Primer Name	Primer Sequence (5' → 3')
GAPDH Fw	GTC AGT GGT GGA CCT GAC CT
GAPDH Rv	TGC TGT AGC CAA ATT CGT TG
RUNX2 Fw	GCA GTT CCC AAG CAT TTC AT
RUNX2 Rv	CAC TCT GGC TTT GGG AAG AG
OCN Fw	GTG CAG AGT CCA GCA AAG GT
OCN Rv	TCA GCC AAC TCG TCA CAG TC
OSX Fw	TAA TGG GCT CCT TTC ACC TG
OSX Rv	CAC TGG GCA GAC AGT CAG AA
OPN Fw	TGA ACC GAG TCA GCT GGA TG
OPN Rv	TGA AAT TCA TGG CTG TGG AA

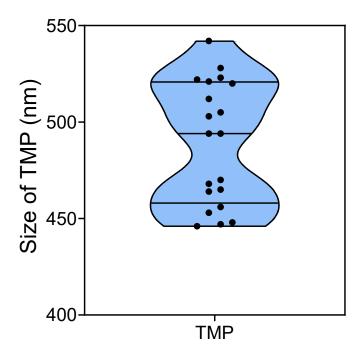


Figure S1. The diameter of TMP measured from SEM images (n = 20).