Supporting Information

Inorganic coatings for optimized non-viral transfection of stem cells

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Fabrication of mineral coatings. Polypropylene 96-well plate and poly (lactide-co-glycolide) (PLG, lactide:glycolide = 85:15, average M_W = 50,000–70,000) were purchased from Sigma-Aldrich (St. Louis, MO). To prepare PLG coated plate, PLG (10 mg) dissolved in acetone (Sigma-Aldrich, St Louis, MO) was transferred in each well (diameter 6.35 mm) and dried. PLG coated plates were exposed to ethylene oxide gas sterilizer to remove residual chemicals and to sterilize the plates. Prior to mineral formation, each well was incubated in NaOH solution (0.5 M) for 30 min for hydrolysis to create a surface rich in COOH and OH groups, then rinsed with distilled (DI) water (18 MΩ cm). Various mineral solutions were prepared by dissolving NaCl, KCl, $MgSO_4$, $MgCl_2$, $NaHCO_3$, $CaCl_2$, KH_2PO_4, and MES (Fisher Scientific, Pittsburgh, PA) in DI water, and pH was adjusted by adding HCl and NaOH solution to make supersaturated mineral solutions (Table S1). Then, all mSBF solutions were filtered through sterile filter in a sterile laminar flow hood. For mineral formation, hydrolyzed PLG in each well was incubated in 2 × mSBF solution with 4.2 mM of CO_3^{2-} ion concentration at 37 °C for 3 d to form precursor mineral coatings. After 3 d mineralization, various mineral solutions were incubated on precursor mineral coatings at 37 °C for 3 d to form various mineral coatings. The solutions were refreshed every 12 h and resulting mineral-coated plates were rinsed in DI water and air dried for characterizing mineral coatings.

Characterization of mineral coatings. FTIR spectrometer (Bruker, model EQUINOX 55) was used for structural analysis. The mineral coatings were mixed with potassium bromide (KBr), pressed into a KBr pellet, and analyzed. All FTIR spectra were recorded in the range of 400–2000 cm^{-1} (Fig. 2a). For calculating crystallinity index, FTIR spectra of each mineral coating were used as previously described [1, 2]. The heights of the absorption peaks at 603 cm^{-1} and 565 cm^{-1} from baseline are summed and divided by the height of the valley between them (Fig. 2b and Supplementary Fig. S1). The crystal phases of mineral coatings were analyzed XRD (Bruker AXS, model HI-STAR). The minerals collected from PLG films were mounted by glass number 50 capillary tubes (Hampton research, Aliso Viejo, CA) and analyzed under Cu Kα radiation. XRD spectra were taken for 10 min scanning in the range of 2θ = 10–50° (Supplementary Fig. S2). To characterize mineral composition, EDS analysis was carried out with the acceleration voltage of 15 kV. The calcium and phosphorus composition of mineral coatings were determined and the ratio of calcium compared to phosphorus was denoted by Ca/P ratio (Fig. 2c). For pore size measurement of mineral coatings, 6 different locations of SEM image from each sample were analyzed by ImageJ software (National Institutes of Health, Bethesda, MD) (Supplementary Fig. S3).

Quantification of naked pDNA and pDNA complex on mineral coatings. After adsorption of pDNA complexes on mineral coatings described in *Methods* section, the amount of bound complexes was calculated by subtracting the amount of complexes that remained in solution from the initially added amount of complexes. pDNA was dissociated from complexes by adding 10 times of sodium oleate (Sigma-Aldrich, St Louis, MO) than the complex amount. After dissociation at 37 °C for 5 h, pDNA amount was determined by adding working solution prepared with the Quant-iT Picogreen dsDNA assay kit (Invitrogen, Carlsbad, CA) (Fig. 3a). For

naked pDNA quantification, solution from the initially added amount of naked pDNA was collected and mixed with working solution of dsDNA assay kit (Supplementary Fig. S6). The fluorescence measured at 520 nm was converted to the amount of pDNA using standard curves prepared with known concentration of dissociated pDNA.

Quantification of Ca^{2+} and PO_4^{3-} concentrations in cell culture medium during transfection. MEM solutions were collected after 12 h of hMSC transfection. To assay for soluble Ca^{2+} concentrations, working solution (0.4 mM Arsenazo III (MP Biomedicals, Solon, OH) in 0.02 M Tris-base at pH 7.4) was mixed with aliquot of each solution. Absorbance at 650 nm was converted to Ca^{2+} concentration using standard curves relating absorbance intensity to Ca^{2+} concentration (Supplementary Fig. S5a). For soluble PO_4^{3-} concentrations, working solution (1 part of 10 mM ammonium molybdate: 1 part of 5 N sulfuric acid: 2 parts of acetone) was mixed with aliquot of each solution. Absorbance at 405 nm was converted to PO_4^{3-} concentration using standard curves relating absorbance intensity to PO_4^{3-} concentration (Supplementary Fig. S5b).

Fabrication of mineral coatings on 3D PLG scaffolds. PLG scaffolds were fabricated in poly(propylene) 96-well plate using PLG (10% w/v) in acetone with salt fusion/solvent casting/salt leaching technique. NaCl particles were previously sieved to 250-425 μm and used as porogen. NaCl (130 mg) was placed in each well of 96-well plate and incubated in 95% humidity cell culture incubator at 37 °C for 4 h for salt fusion. Then the fused salt template was dried in an oven at 50 °C for overnight. PLG solution (30 μL) was added into each well and the whole plate was centrifuged at 2000 RPM to wet all the NaCl particles. After evaporating the solvent, the whole plate was then immersed in beaker filled with DI water (4.0 L) to leach the salt particles out. The water was refreshed every 4 h and the leaching process took approximately 48 h. To coat the PLG scaffolds with mineral, the scaffold was treated by NaOH

(200 μL, 0.1 M) for 5 min at room temperature to activate the COOH and OH group on polymer surface. Various mSBF solutions (200 μL) **(**Table S1**)** was then added into each well of scaffolds after extensively washing out the NaOH residue. The mSBF solution was renewed every 12 h to maintain a consistent ionic strength for 7 days. The morphology of the PLG scaffold before and after mineral coatings was observed using SEM as stated in *Methods* Section. Mineral coated PLG scaffold was stained using Alizarin Red S (Sigma-Aldrich, St Louis, MO) for identify calcium on the scaffold (Supplementary Fig. S10).

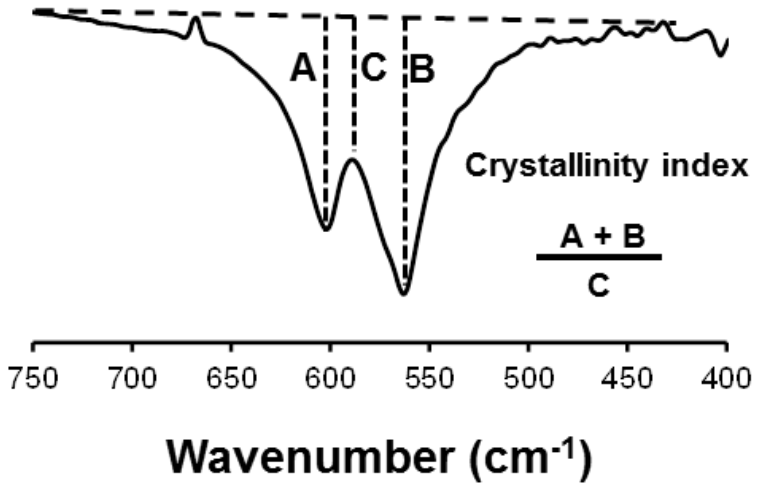
hMSC transfection on mineral coated 3D PLG scaffolds. The procedure for the transfection was similar to that of mineral coatings on PLG surfaces with some adjustments: pDNA (1 μg) was used for each scaffold; pDNA binding was conducted in pDNA/Lipofectamine 2000 complex containing medium (100 μL); hMSC was seeded on the mineral coated scaffolds with complexes at a density of 1.0×10^5 cells/scaffold. After 2 d of cell culture on mineral scaffolds, each medium was taken for luciferase activity assay and total protein amount on each scaffold was measured by micro BCA kit (Thermo Scientific, Rockford, IL) (Supplementary Fig. S11a). Also, luminescence per well was imaged using in vivo imaging system (IVIS) (Caliper Life Sciences, Mountain view, CA) (Supplementary Fig. S11b).

References

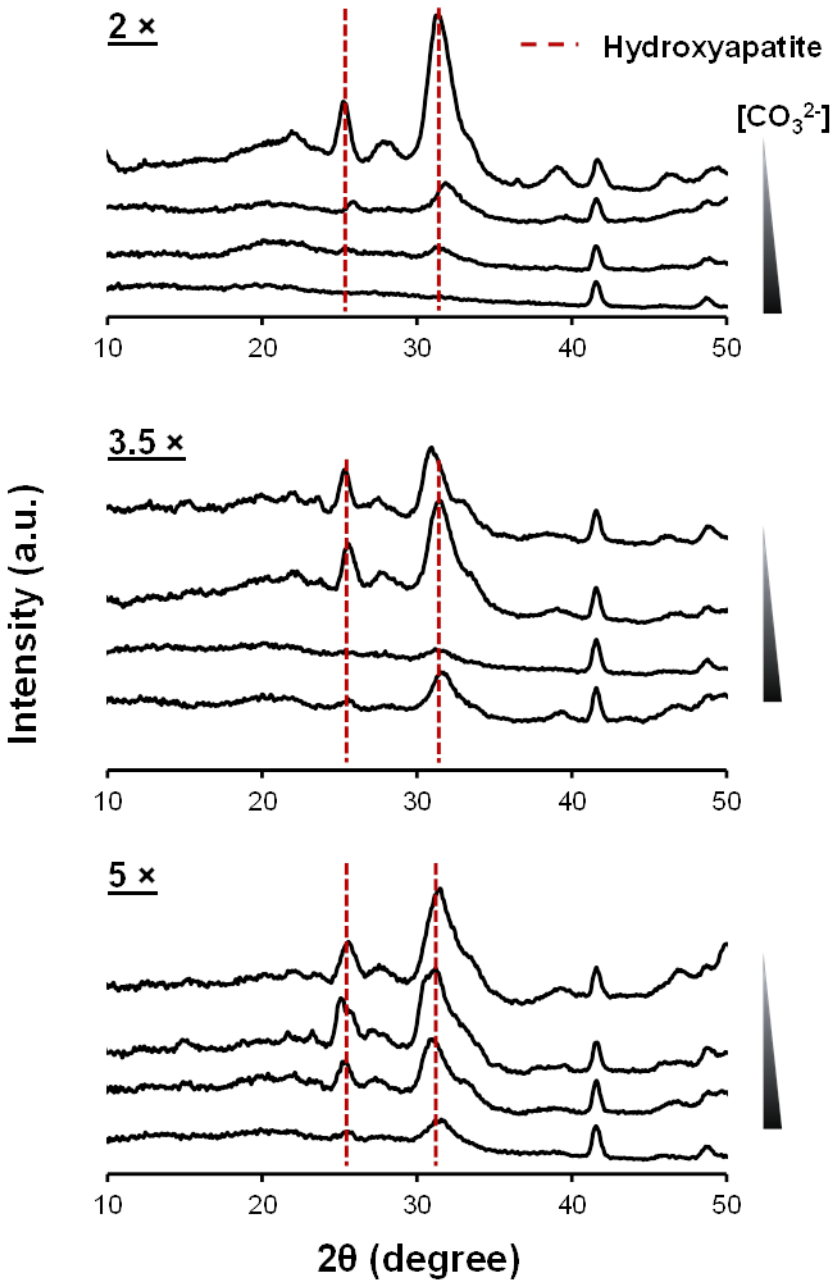
[1] P. D. Frazier, M. F. Little, F. S. Casciani, *Arch. Oral. Biol.* **1967**, 12, 35.
[2] D. Farlay, G. Panczer, C. Rey, P. D. Delmas, G. Boivin, *J. Bone. Miner. Metab.* **2010**, 28, 433.

Table S1. Ion concentrations of mSBF solutions.

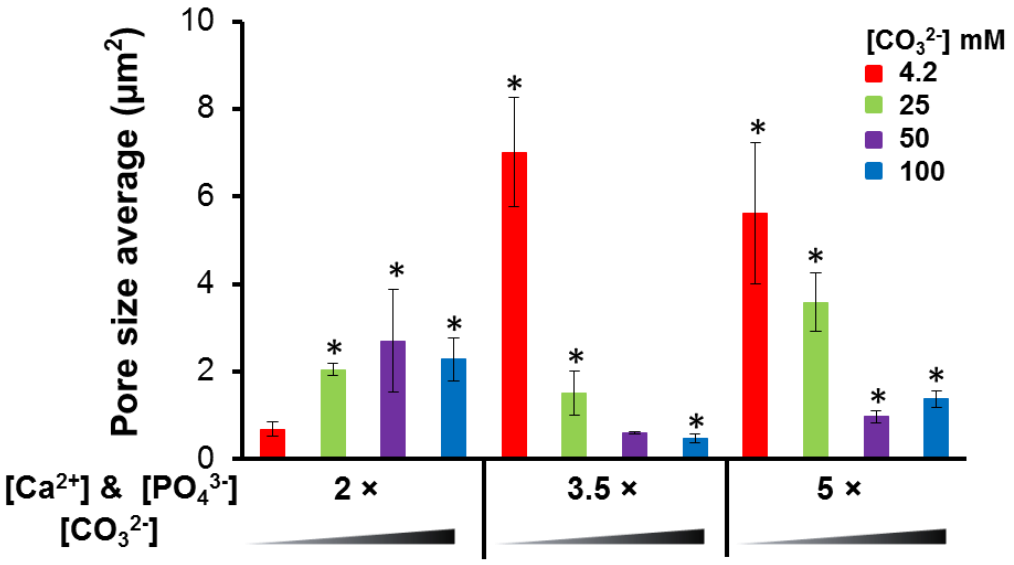
	SBF	mSBF		
$\frac{[Ca^{2+}] \& [PO_4^{3-}]}{[Ca^{2+}] \& [PO_4^{3-}]_{\text{in blood plasma}}}$	1 ×	2 ×	3.5 ×	5 ×
Ca^{2+} [mM]	2.5	5	8.8	12.5
PO_4^{3-} [mM]	1	2	3.5	5
CO_3^{2-} [mM]	4.2	4.2, 25, 50, 100	4.2, 25, 50, 100	4.2, 25, 50, 100
pH	7.4	6.8	6.1	5.8



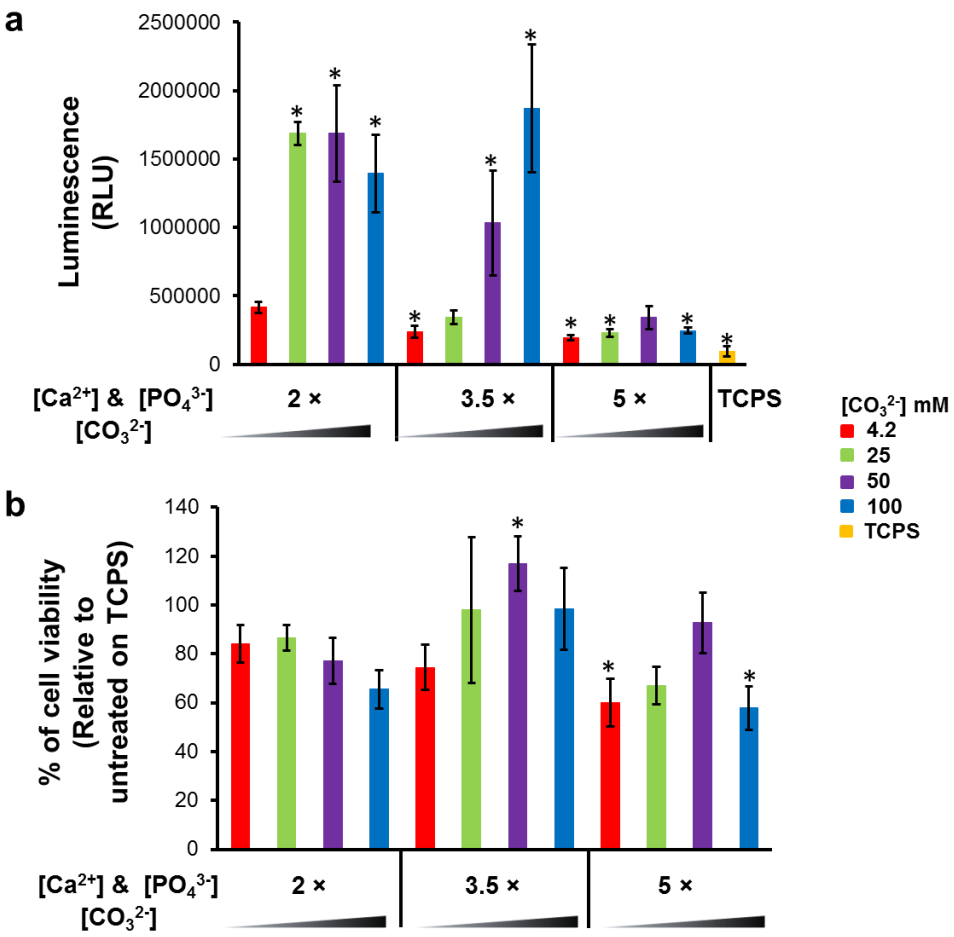
Supplementary Figure S1 | Crystallinity index. Schematic representation of calculating crystallinity index. The heights of the absorption peaks at 603 cm^{-1} and 565 cm^{-1} from baseline are summed and divided by the height of the valley between them.



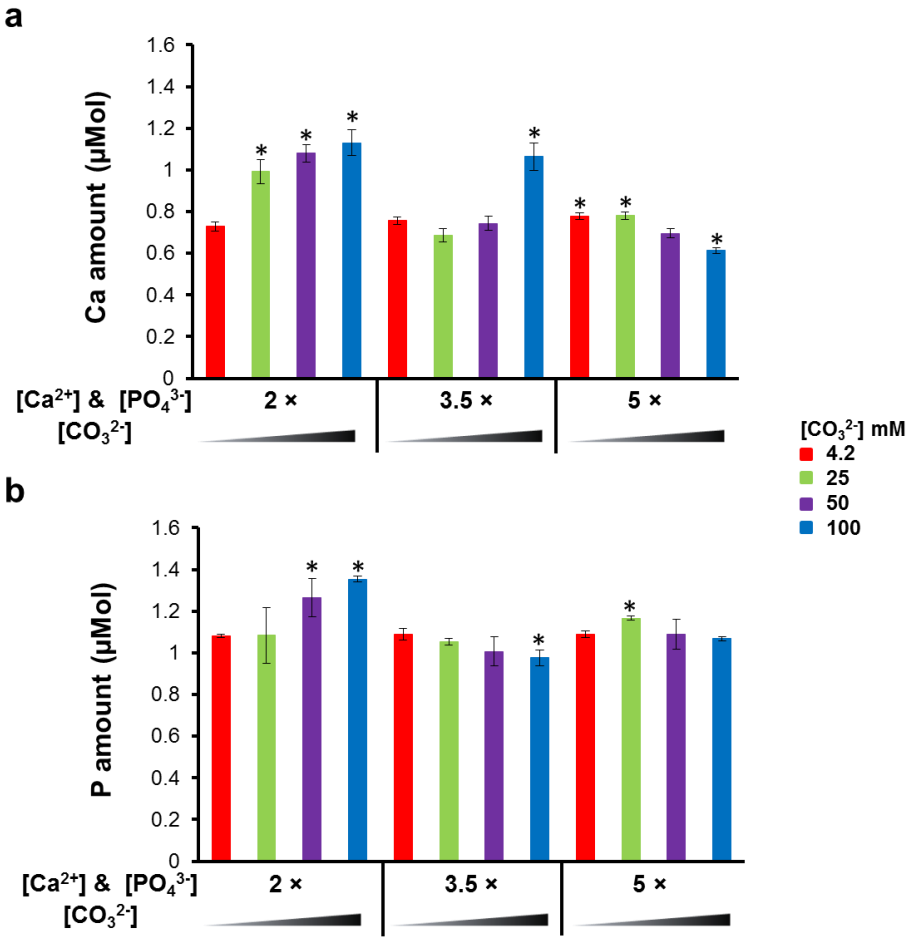
Supplementary Figure S2 | XRD spectra of CaP mineral coatings. Mineral coatings were grown in mSBF solutions for 3 days after precursor mineral layer formation. The peaks at 26° and 31° correspond to planes of HA (···).



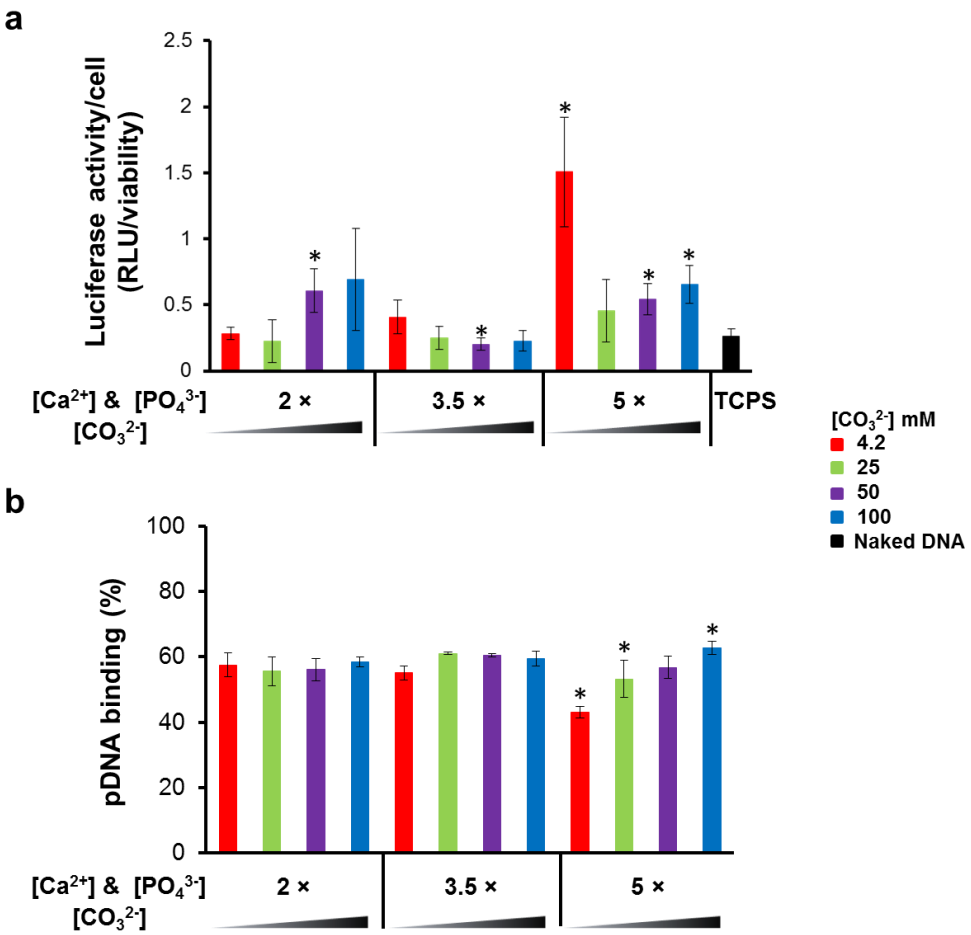
Supplementary Figure S3 | Pore size of mineral coatings. Pore size area was measured from imageJ software using SEM images of mineral coatings. * indicated significant difference compared to 2 × mSBF with 4.2 mM of CO_3^{2-} (red bar at far left) by Student's *t*-test with $p < 0.05$. Date represents mean ± SD ($n = 6$).



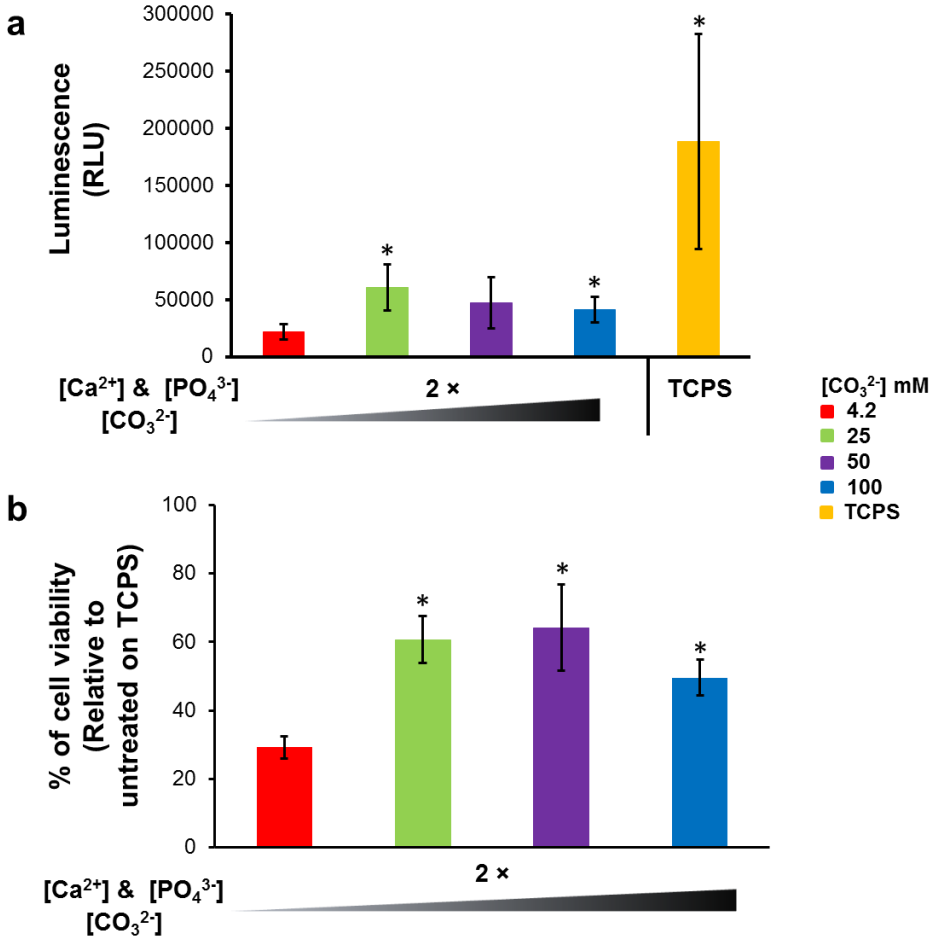
Supplementary Figure S4 | Luciferase activity and cell viability after hMSC transfection. (a) Luminescence and (b) cell viability of hMSCs after 2 days culture on mineral coatings incubated with 3.2 µg of pDNA complexes. To compare with a standard transfection method, soluble pDNA complexes were also added to hMSCs on tissue culture polystyrene (TCPS). * indicates significant difference compared to 2 × mSBF with 4.2 mM of CO_3^{2-} (red bar at far left) by Student's *t*-test with $p < 0.05$. Data represents mean ± SD ($n = 4$).



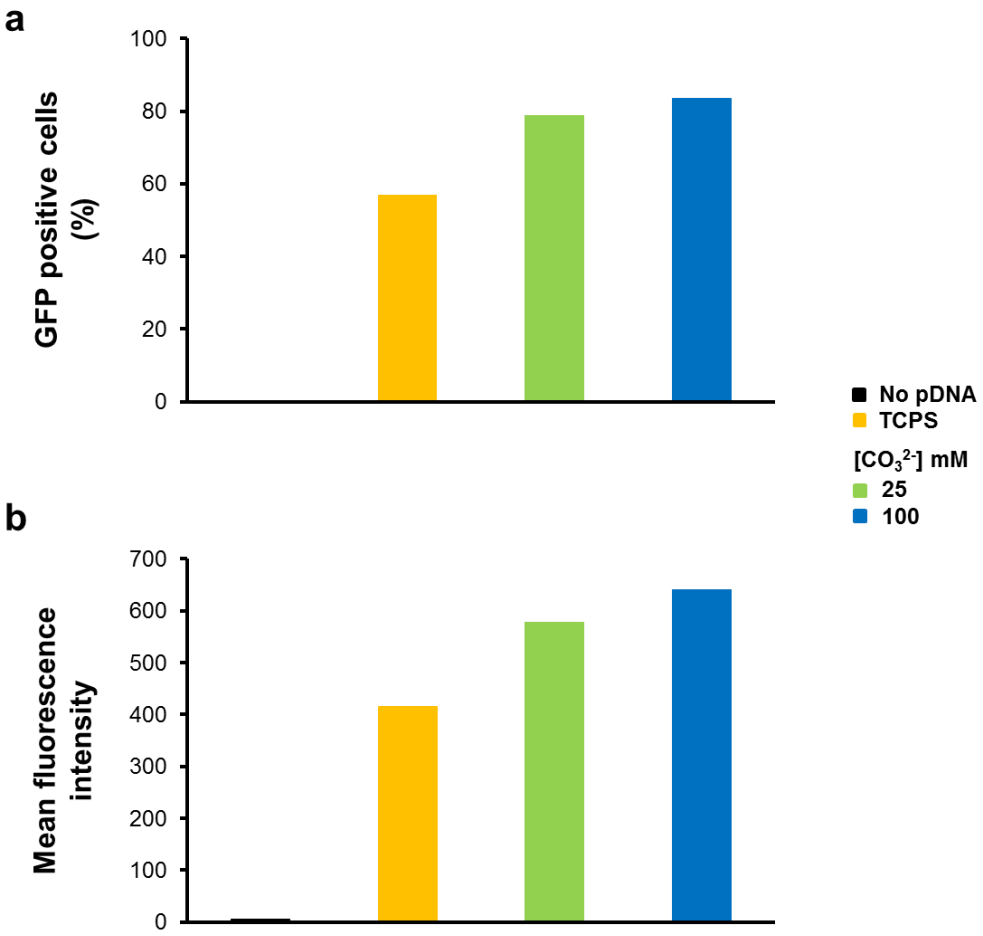
Supplementary Figure S5 | Dissolution of mineral coatings during hMSC transfection. (a) Ca^{2+} and (b) PO_4^{3-} amount in cell culture medium were measured after 12 h of hMSC transfection on mineral coatings. * indicated significant difference compared to 2 × mSBF with 4.2 mM of CO_3^{2-} (red bar at far left) by Student's *t*-test with $p < 0.05$. Date represents mean ± SD ($n = 4$).



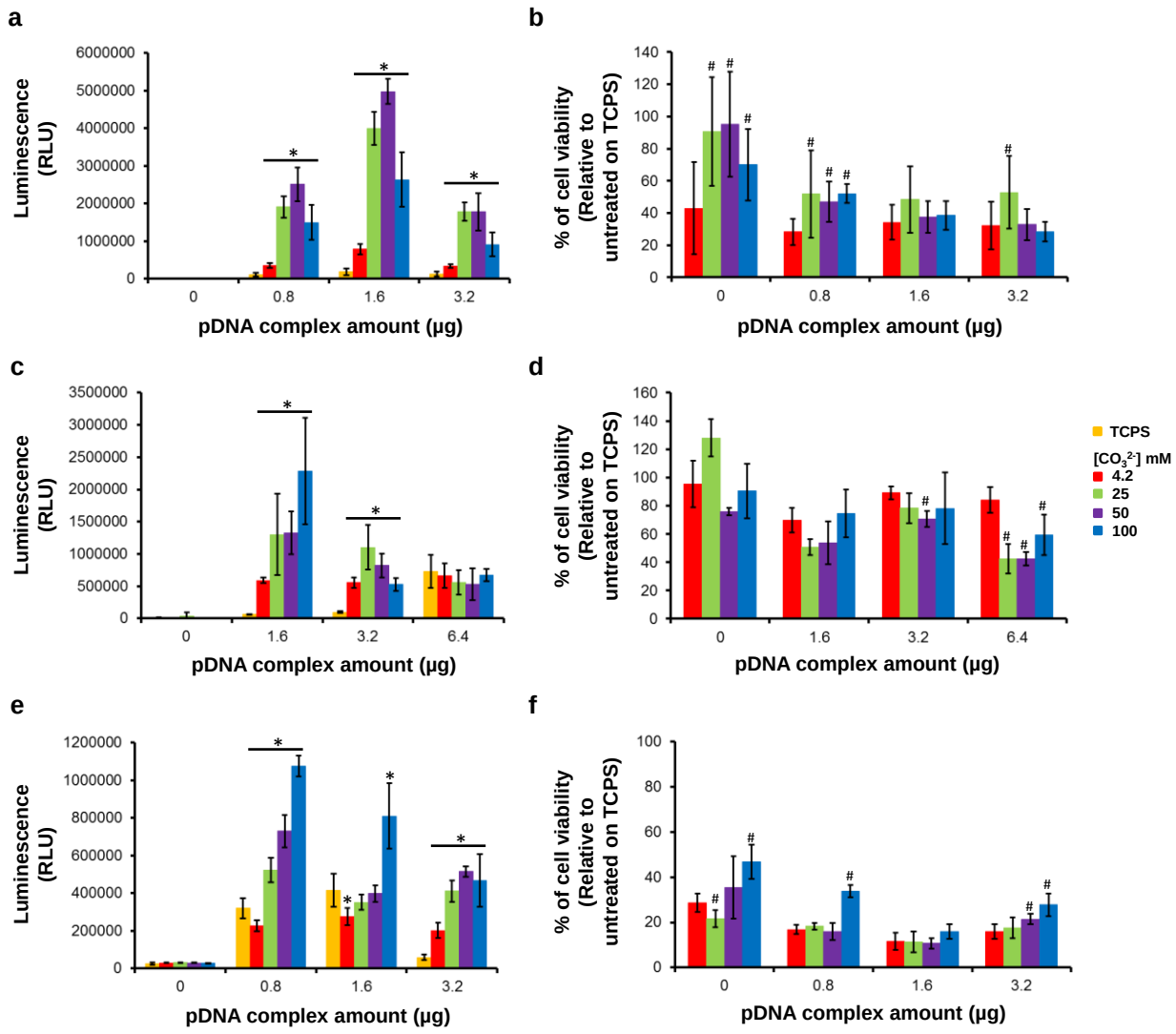
Supplementary Figure S6 | Screening of hMSC transfection on mineral coatings with naked pDNA. (a) Luciferase activity of hMSCs after 2 days culture on mineral coatings containing naked pDNA. To compare with a standard transfection method, soluble naked pDNA was also added to hMSCs on tissue culture polystyrene (TCPS). (b) Naked pDNA binding on mineral coatings. The binding of the naked pDNA was measured after 5 h incubation on the mineral coatings. * indicated significant difference compared to 2 × mSBF with 4.2 mM of CO_3^{2-} (red bar at far left) by Student's *t*-test with $p < 0.05$. Date represents mean ± SD ($n = 4$).



Supplementary Figure S7 | Luciferase activity and cell viability after solution based hMSC transfection on mineral coatings. (a) Luminescence and (b) cell viability of hMSCs after 2 days culture on mineral coatings. pDNA complexes were added into cell culture medium after 1 h of cell seeding on mineral coatings formed in 2 × mSBF with various [CO_3^{2-}]. For a control, hMSCs were grown on TCPS. * indicates significant difference compared to 2 × mSBF with 4.2 mM of CO_3^{2-} (red bar at far left) by Student's *t*-test with $p < 0.05$. Data represents mean ± SD (*n* = 6).

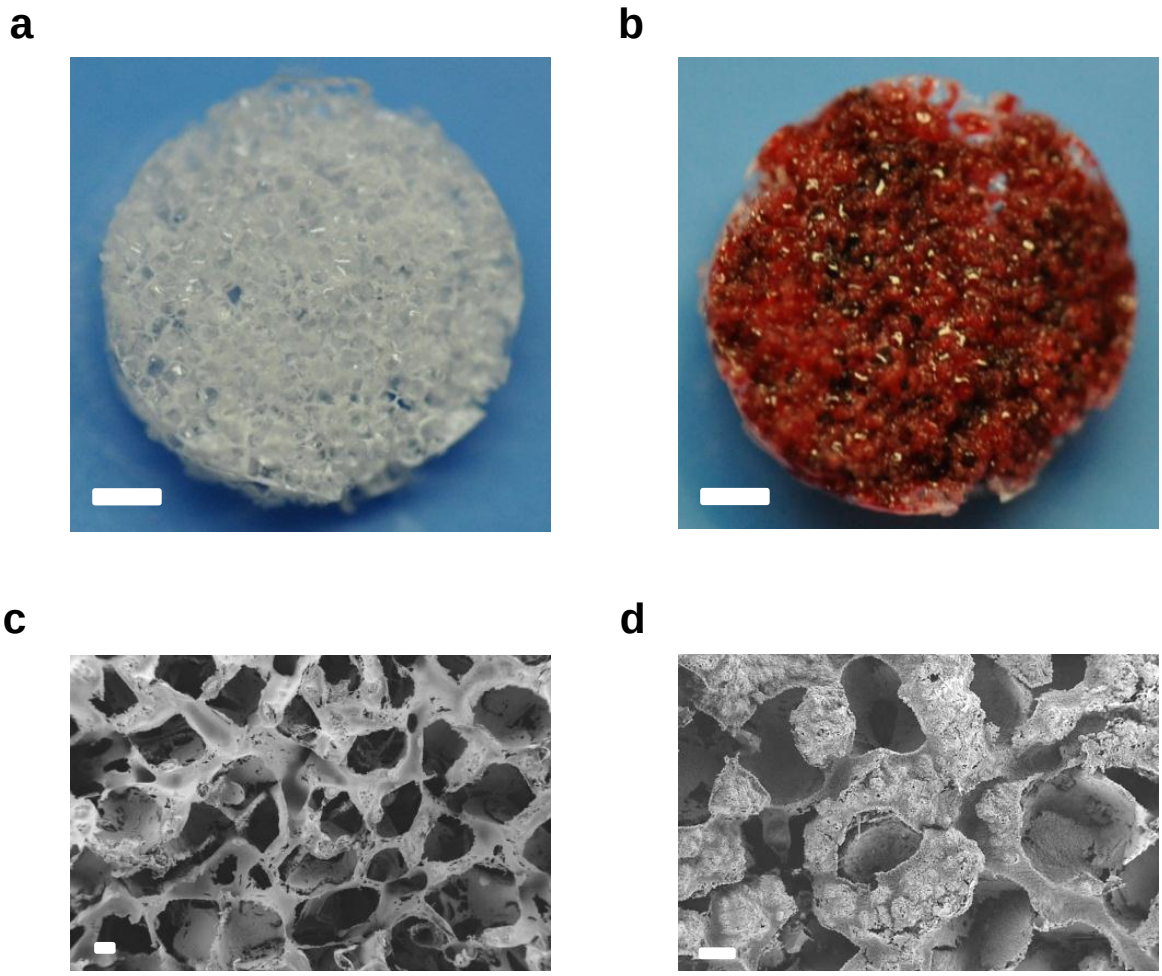


Supplementary Figure S8 | Analysis of GFP-positive cells after hMSC transfection on mineral coatings. Percentage of GFP-positive cells and mean fluorescence intensity of GFP-positive cells after transfection on mineral coatings formed in 2 × mSBF with various [CO_3^{2-}] and TCPS using pEGFP complexes.

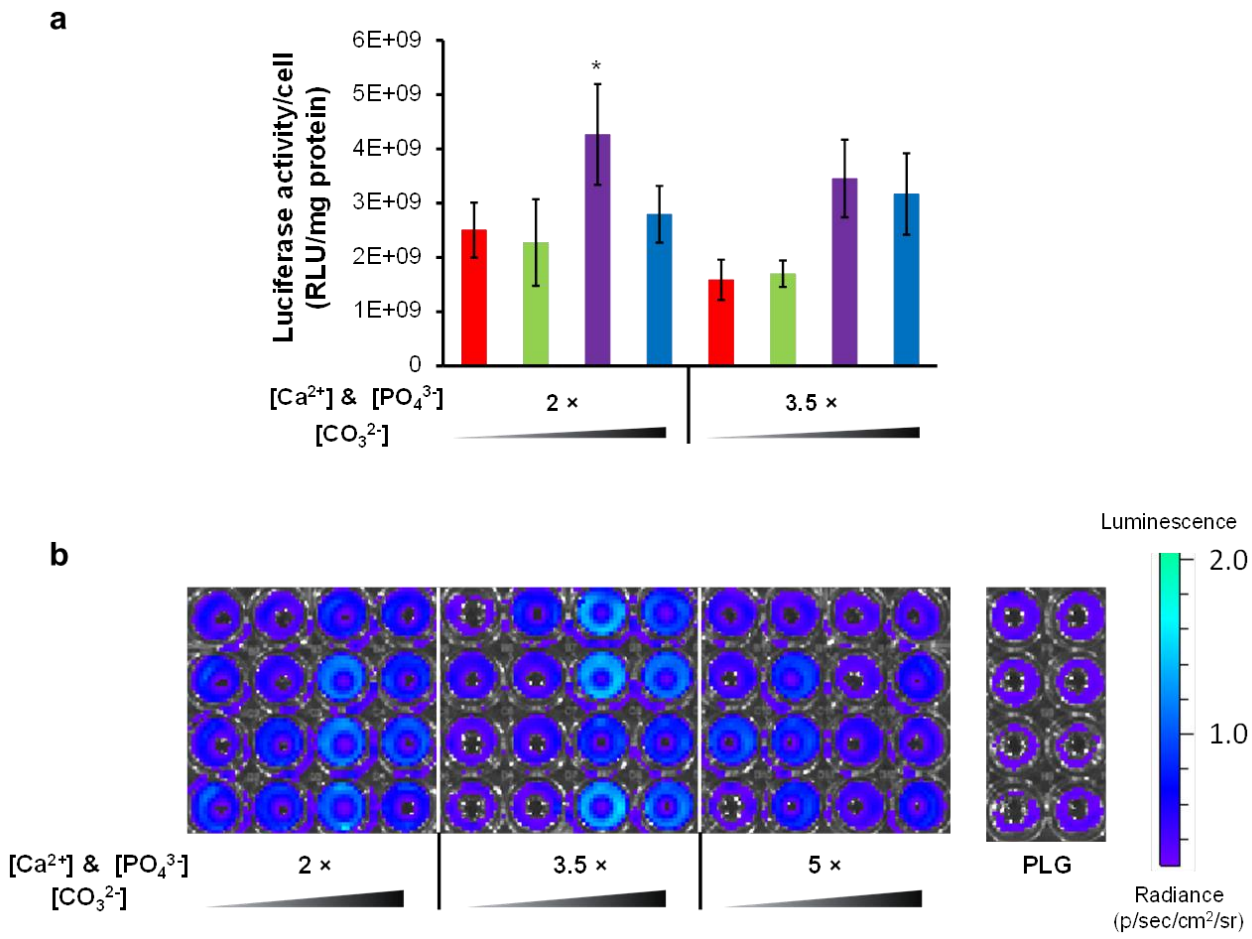


Supplementary Figure S9 | Luciferase activity and cell viability of multiple cell types. (a, c, e) Luminescence and (b, d, f) cell viability of hMSCs (a and b), C3H10T1/2 cells (c and d) and HUVECs (e and f) after 2 days culture on mineral coatings incubated with various pDNA complex amount. Specified amounts of pDNA complexes were adsorbed on mineral coatings formed in 2 × mSBF with various [CO_3^{2-}]. Luciferase activity was measured after 2 days of transfection of hMSCs (n = 6), C3H10T1/2 cells (n = 3), and HUVECs (n = 6). * indicates significant difference compared to TCPS condition by Student's t-test with $p < 0.05$. # indicates

significant difference compared to 2 × mSBF with 4.2 mM of CO_3^{2-} (red bar at far left) by Student's *t*-test with $p < 0.05$.

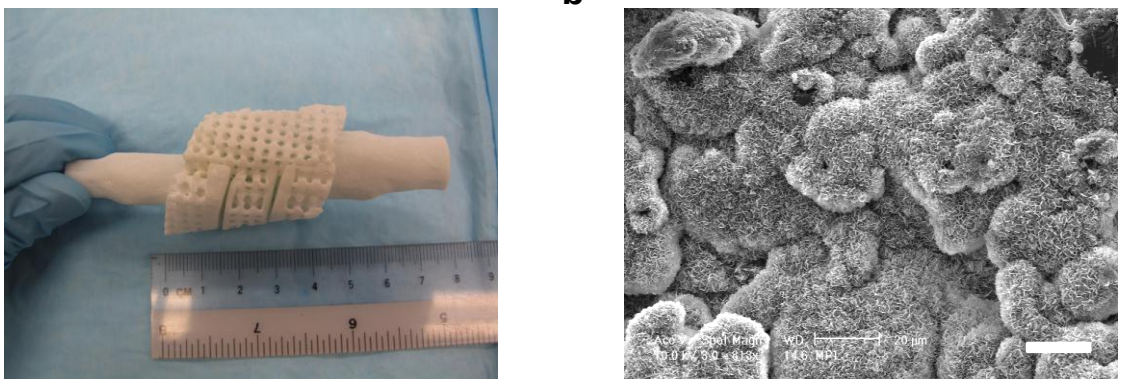


Supplementary Figure S10 | Mineral coating formation on 3-dimensional PLG scaffolds. Photograph images (a, b) and SEM images (c, d) of scaffolds before mineralization (a, c) and after mineralization (b, d). Scale bars, 1 mm for photograph images and 100 μm for SEM images.



Supplementary Figure S11 | hMSC transfection on mineral coated 3-dimentional PLG scaffolds. (a) Luciferase activity of hMSCs after 2 days culture on mineralized 3-dimensional PLG scaffolds containing pDNA complexes. * indicates significant difference compared to CaP mineral coating grown in 2 × mSBF with 4.2 mM of CO_3^{2-} (red bar at far left) by Student's *t*-test with $p < 0.05$. Data represents mean ± SD ($n = 4$). (b) Luminescence intensity from cell culture medium after 2 days of hMSC transfection on mineral coatings and PLG..

a **b**



Supplementary Figure S12 | Mineral coating formation on PCL scaffold for a pig mandible reconstruction. Photograph image (a) and SEM image (b) of scaffold after mineralization. Modular polycaprolactone (PCL) scaffold, which is multiple centimeters in scale, was mineral coated using mSBF solution. Scale bars, 20 μm for SEM image.