Supporting information

Effect of strontium substitution on functional activity of phosphate-based glass

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Table S1. The sequence of primer used in qPCR in this study

Molecules	Primer sequences (5'- 3')	Product size (bp)	Accession number from NCBI database
RUNX2	Forward: GGGAACCAAGAAGGCACAGA	152	NM_001271627.1
	Reverse: ACTTGGTGCAGAGTTCAGGG		
OCN	Forward: TTGGCCCAGACCTAGCAGA	100	NM_007541.3
	Reverse: CTGGGCTTGGCATCTGTGA		
OSX	Forward: GTCCTCTCTGCTTGAGGAAGAA	131	NM_130458.4
	Reverse: TCTTTGTGCCTCCTTTCCCC		
BSP	Forward: CGGTTTCCAGTCCAGGGAGG	174	NM_008318.3
	Reverse: CGAGAGTGTGGAAAGTGTGGA		
TNF-α	Forward: TGTAGCCCACGTCGTAGCAAA	197	NM_013693.3
	Reverse: TGTGGGTGAGGAGCACGTA		
IL-1β	Forward: TGCCACCTTTTGACAGTGATG	136	NM_008361.4
	Reverse: ATGTGCTGCTGCGAGATTTG		
IL-6	Forward: TCTTGGGACTGATGCTGGTG	178	NM_001314054.1
	Reverse: TTGCCATTGCACAACTCTTTTC		
CXCL10	Forward: ATGACGGGCCAGTGAGAATG	188	NM_021274.2
	Reverse: AGGAGCCCTTTTAGACCTTTTT		
NOS2	Forward: GGTGAAGGGACTGAGCTGTT	103	NM_010927.1
	Reverse: ACGTTCTCCGTTCTCTTGCAG		
CD80	Forward: CACCTGGGAAAAACCCCCA	105	NM_001359898.1
	Reverse: ATGACAACGATGACGACGACT		
GAPDH	Forward: CCCACTCTTCCACCTTCGATG	201	NM_001289726.1
	Reverse: CGAGTTGGGATAGGGCCTCT		

Table S2. The composition of BG used in this study

	SiO_2	CaO	Na_2O	P_2O_5
BG	46.1 mol%	26.9 mol%	24.4 mol%	2.6 mol%

BG; 45S5 bioactive glass

To obtain the BG, SiO_2 (Sigma Aldrich), $CaCO_3$ (Sigma Aldrich), Na_2CO_3 (Sigma Aldrich), P_2O_5 (Sigma Aldrich) were used. The composition of fabricated BG is summarized in Table S2. The mixture was placed in alumina crucible and heated to an intermediate step at 900 °C for 90 min to remove the carbon dioxide and impurity, followed by a second step at 1350 °C for 4h followed by quenching at 25 °C.

Table S3. Energy dispersive spectrometry of PBG and PSr samples

Unit: Atomic %

Code	P	Ca	Na	Sr
PBG	63.41	17.58	19.01	0.00
PSr1.5	62.69	15.53	20.94	0.84
PSr3	63.17	15.61	19.59	1.63
PSr6	63.94	12.94	19.85	3.27
PSr15	64.20	8.28	19.98	7.54
PSr30	67.95	0.00	17.70	14.35

P; Phosphorous; Ca; Calcium; Na; Sodium; Sr; Strontium.

PBG was detected in P, Ca, and Na. PSr samples ranging from 1.5 to 15 mol% SrO were detected to P, Ca, Na, and Sr atoms. According to EDS analysis, it is successfully fabricated to PBG and PSr samples. PBG; Phosphate-based glass; PSr; strontium-substituted PBG.

Table S4. pH measurement of PBG and PSr for 28 days

Code	Day 1	Day 7	Day 14	Day 21	Day 28
PBG	4.83 ± 0.06^{a}	4.51 ± 0.01^{a}	4.32 ± 0.04^{a}	4.23 ± 0.05^{a}	4.25 ± 0.01^{a}
PSr1.5	5.82 ± 0.60^{b}	4.51 ± 0.01^{a}	4.21 ± 0.05^{a}	4.21 ± 0.05^{a}	4.22 ± 0.03^{a}
PSr3	6.38 ± 0.13^{bc}	4.53 ± 0.01^{a}	4.25 ± 0.01^{a}	4.22 ± 0.01^{a}	4.23 ± 0.01^{a}
PSr6	6.46 ± 0.13^{bc}	4.60 ± 0.01^{b}	4.26 ± 0.04^{b}	4.22 ± 0.01^{a}	4.23 ± 0.03^{a}
PSr15	6.74 ± 0.05^{c}	4.76 ± 0.04^{c}	4.37 ± 0.01^{c}	4.24 ± 0.04^{a}	4.25 ± 0.03^{a}
PSr30	6.99 ± 0.15^{c}	5.79 ± 0.03^{d}	5.28 ± 0.03^{d}	4.90 ± 0.03^{b}	4.79 ± 0.01^{b}
p-value	4.6×10^{-6}	1.1×10^{-16}	2.2×10^{-13}	6.1×10^{-11}	2.2×10^{-13}

Data represented the mean and standard deviation and performed a one-way analysis of variance (ANOVA) with Tukey's post hoc test at a confidence level of 95%. The different small letter was a significant difference. PBG; phosphate-based glass; PSr; strontium-substituted PBG.

Table S5. Detection of P, Ca, and Sr ion release of PBG and PSr groups on 1 day

Unit: ng/μL

Day 1	P	Ca	Sr
PBG	226.013 ± 0.335^{f}	78.757 ± 0.247^{e}	-
PSr1.5	219.297 ± 1.933^{e}	67.717 ± 0.289^{d}	7.773 ± 0.035^{a}
PSr3	209.913 ± 1.062^{d}	63.947 ± 0.267^{c}	15.307 ± 0.068^{b}
PSr6	190.760 ± 0.317^{c}	50.033 ± 0.260^{b}	25.990 ± 0.141^{c}
PSr15	171.607 ± 0.438^{b}	29.800 ± 0.078^{a}	42.700 ± 0.135^{d}
PSr30	58.620 ± 0.433^{a}	-	57.790 ± 0.050^{e}
p-value	6.2×10^{-22}	2.8×10^{-19}	1.4×10^{-23}

Data represented the mean and standard deviation and performed a one-way analysis of variance (ANOVA) with Tukey's post hoc test at a confidence level of 95%. The different small letter was a significant difference. PBG; phosphate-based glass; PSr; strontium substituted PBG.

Table S6. Cumulative ion release of P, Ca, and Sr ion for PBG and PSr groups on 7 days

Unit: ng/μL

Day 7	P	Ca	Sr
PBG	287.703 ± 0.712^{e}	104.683 ± 0.359^{e}	-
PSr1.5	288.990 ± 2.222^{e}	94.473 ± 0.473^{d}	10.500 ± 0.061^{a}
PSr3	279.280 ± 1.259^{d}	88.817 ± 0.294^{c}	20.670 ± 0.080^{b}
PSr6	262.400 ± 1.700^{c}	73.107 ± 0.405^{b}	37.343 ± 0.220^{c}
PSr15	234.123 ± 0.481^{b}	40.833 ± 0.072^{a}	74.513 ± 0.217^{d}
PSr30	105.213 ± 0.675^{a}	-	79.350 ± 0.095^{e}
p-value	7.7×10^{-21}	5.4×10^{-19}	1.8×10^{-23}

Data represented the mean and standard deviation and performed a one-way analysis of variance (ANOVA) with Tukey's post hoc test at a confidence level of 95%. The different small letter was a significant difference. PBG; phosphate-based glass; PSr; strontium-substituted PBG.

Table S7. Cumulative ion release of P, Ca, and Sr ion for PBG and PSr groups on 14 days

Unit: ng/μL

Day 14	P	Ca	Sr
PBG	292.400 ± 0.882^{d}	107.200 ± 0.346^{e}	-
PSr1.5	296.543 ± 2.174^{e}	97.930 ± 0.529^{d}	10.920 ± 0.070^{a}
PSr3	288.880 ± 1.354^{d}	93.023 ± 0.378^{c}	21.730 ± 0.105^{b}
PSr6	277.503 ± 1.822^{c}	78.477 ± 0.418^{b}	40.257 ± 0.226^{c}
PSr15	251.900 ± 0.642^{b}	44.307 ± 0.051^{a}	81.650 ± 0.173^{d}
PSr30	143.357 ± 2.379^{a}	-	95.727 ± 0.313^{e}
p-value	5.3×10^{-20}	1.8×10^{-18}	4.4×10^{-23}

Data represented the mean and standard deviation and performed a one-way analysis of variance (ANOVA) with Tukey's post hoc test at a confidence level of 95%. The different small letter was a significant difference. PBG; phosphate-based glass; PSr; strontium-substituted PBG.

Table S8. Cumulative ion release of P, Ca, and Sr ion for PBG and PSr group on 21 days

Unit: ng/μL

Day 21	P	Ca	Sr
PBG	295.483 ± 1.004^{d}	108.417 ± 0.357^{e}	-
PSr1.5	300.433 ± 1.963^{e}	99.390 ± 0.010^{d}	11.110 ± 0.070^{a}
PSr3	295.693 ± 1.316^{d}	94.877 ± 0.363^{c}	22.207 ± 0.104^{b}
PSr6	285.167 ± 2.061^{c}	80.843 ± 0.355^{b}	41.593 ± 0.200^{c}
PSr15	264.277 ± 0.656^{b}	45.943 ± 0.040^{a}	85.480 ± 0.131^{d}
PSr30	143.357 ± 2.379^{a}	-	105.617 ± 0.306^{e}
p-value	8.0×10^{-19}	1.2×10^{-18}	7.8×10^{-24}

Data represented the mean and standard deviation and performed to one-way analysis of variance (ANOVA) with Tukey's post hoc test at a confidence level of 95%. The different small letter was a significant difference. PBG; phosphate-based glass; PSr; strontium-substituted PBG.

Table S9. Cumulative ion release of P, Ca, and Sr ion for PBG and PSr group on 28 days

Unit: ng/μL

Day 28	P	Ca	Sr
PBG	296.320 ± 1.189^{d}	109.263 ± 0.067^{a}	-
PSr1.5	302.147 ± 1.862^{e}	100.490 ± 0.608^{d}	11.260 ± 0.078^{a}
PSr3	298.040 ± 1.083^{de}	95.953 ± 0.377^{c}	22.517 ± 0.114^{a}
PSr6	289.703 ± 1.658^{c}	82.520 ± 0.426^{b}	42.647 ± 0.242^{b}
PSr15	269.007 ± 0.774^{b}	47.017 ± 0.029^{a}	88.470 ± 0.147^{c}
PSr30	154.413 ± 2.681^{a}	-	114.267 ± 0.193^{d}
p-value	1.5×10^{-18}	4.9×10^{-18}	1.3×10^{-24}

Data represented the mean and standard deviation and performed a one-way analysis of variance (ANOVA) with Tukey's post hoc test at a confidence level of 95%. The different small letter was a significant difference. PBG; phosphate-based glass; PSr; strontium substituted PBG.

Table S10. The *p*-value of in vitro qPCR assay for preosteoblast from PSr6 against benchmarking BG

	RUNX2	OCN	OSX	BSP
Day 3 of the *p*-value	0.026	0.002	0.081	0.001
Day 7 of the *p*-value	0.009	0.001	0.041	0.005

RUNX2: runt-related transcription factor 2, OCN: osteocalcin; OSX: osterix, BSP: bone sialoprotein, and OPN-osteopontin. BG; 45S5 bioactive glass, PSr6; 6 mol% strontium-substituted PBG.

Table S11. The *p*-value of in vitro qPCR assay for macrophage from PSr6 against benchmarking BG

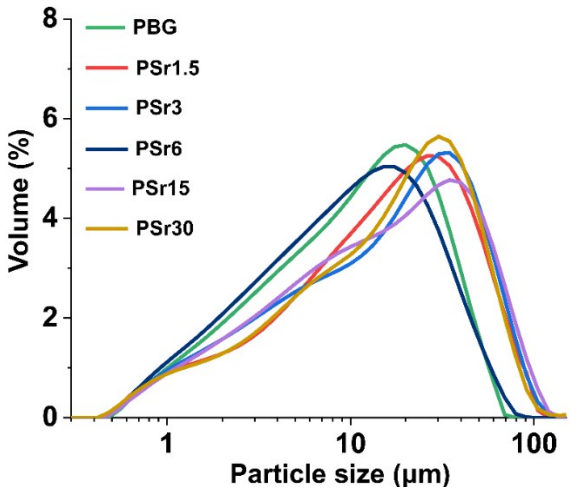
	TNF-α	IL-1β	IL-6	CXCL10	NOS2	CD80
p-value	0.018	0.012	0.012	0.02	0.001	0.007

TNF-α: tumor necrosis factor-alpha, IL-1β: interleukin 1 beta, IL-6: interleukin 6, CXCL10: chemokine ligand (C-X-C motif) 10, NOS2: nitric oxide synthase 2, CD80: cluster of differentiation 80. BG; 45S5 bioactive glass, PSr6; 6 mol% strontium-substituted PBG.

Table S12. Histomorphometric results from benchmarking PSr6 against BG

	BG		PSr6	
	Area (mm^2)	Coverage (%)	Area (mm^2)	Coverage (%)
NB	2.20 ± 0.91	10.46 ± 4.38	1.83 ± 1.00	11.35 ± 7.38
RBS	8.65 ± 2.84	38.79 ± 3.56	6.14 ± 1.79	36.96 ± 3.20
Others	11.36 ± 3.71	50.75 ± 5.07	8.94 ± 2.36	52.69 ± 7.27

NB; new bone, RBS; residual bone substitute, BG; 45S5 bioactive glass, PSr6; 6 mol% strontium-substitution phosphate-based glass.



Group	D_{50}
PBG	10.53 μm
PSr1.5	15.17 μm
PSr3	15.77 μm
PSr6	9.35 μm
PSr15	14.70 μm
PSr30	16.44 μm

Fig. S1 Particle distribution of PBG and PSr groups. PBG; PBG; Phosphate-based glass, PSr; Strontium-substituted PBG, D_{50}; the mean particle size of a material.

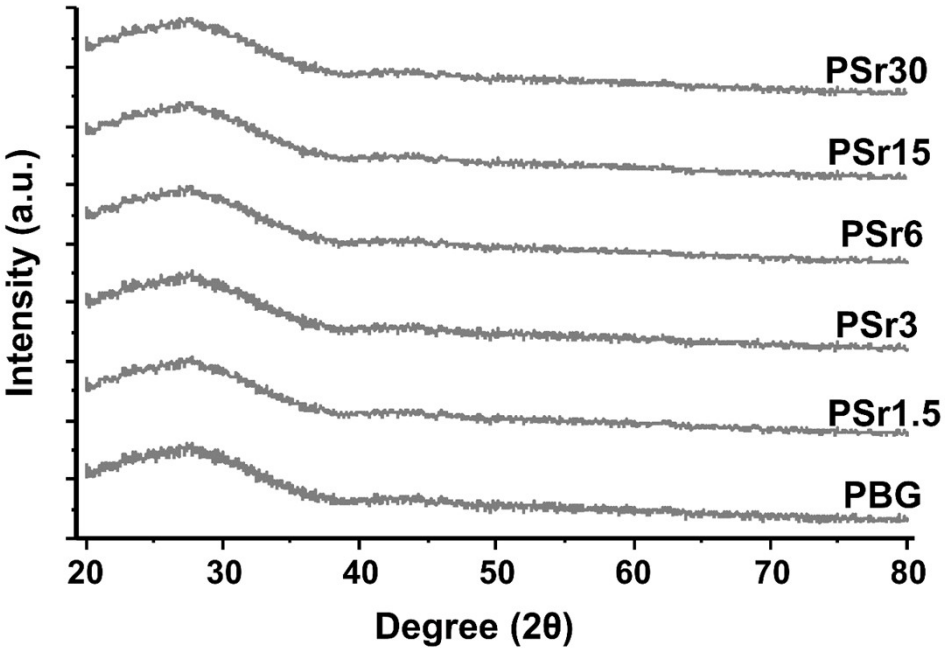


Fig. S2 X-ray diffraction analysis of the phosphate-based glass groups synthesized with strontium substitution up to 30 mol%.

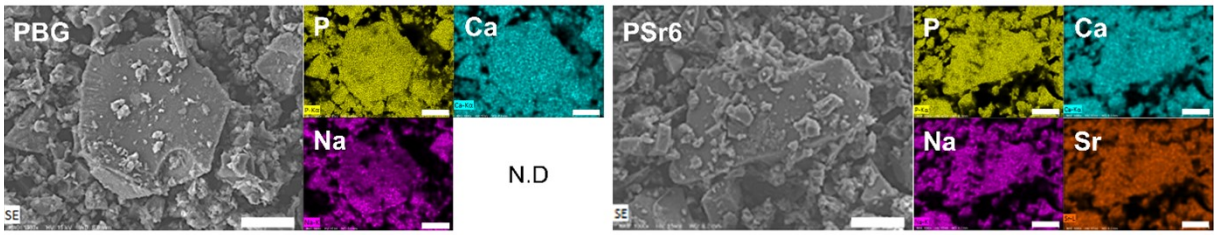


Fig. S3 SEM images and P, Ca, Na, and Sr element distribution of PBG and PSr6 groups. All of the scale bar is 20 μm. PBG; phosphate-based glass, PSr6; 6 mol% strontium-substituted PBG. N.D; Not detected, SEM; Scanning electron microscopy, P; phosphorous, Ca; calcium, Na; sodium, Sr; strontium.

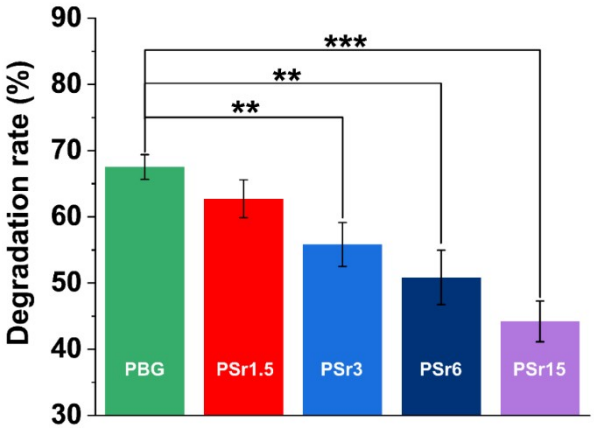


Fig. S4 The degradation rate of PBG and PSr6 groups for 5 days in Tris-HCl buffer solution ($n = 3$). ** $p < 0.01$; *** $p < 0.001$. PBG; phosphate-based glass, PSr; strontium-substituted PBG.

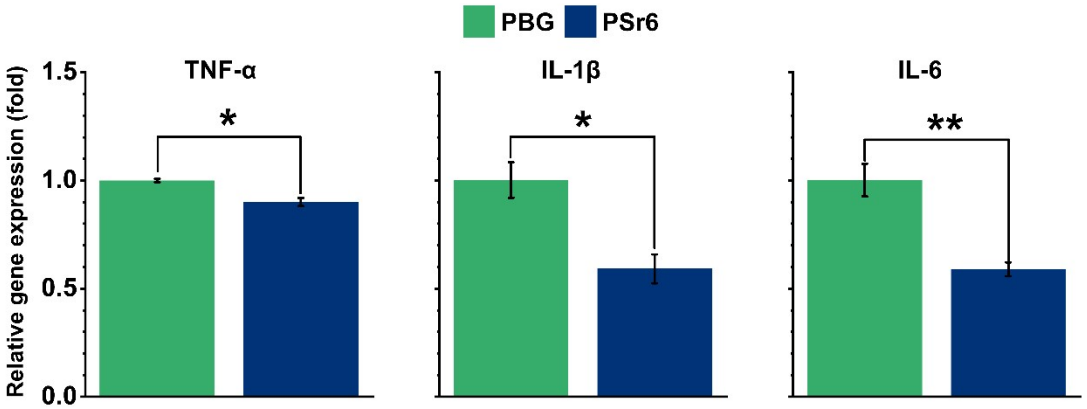


Fig. S5 *In vitro* qPCR assay of TNF-α, IL-1β, and IL-6 with PBG and PSr6 groups ($n = 3$). * $p < 0.05$; ** $p < 0.01$. TNF-α; tumor necrosis factor, IL-1β: interleukin-1beta, IL-6; interleukin-6, PBG; phosphate-based glass, PSr6; 6 mol% strontium-substituted PBG.

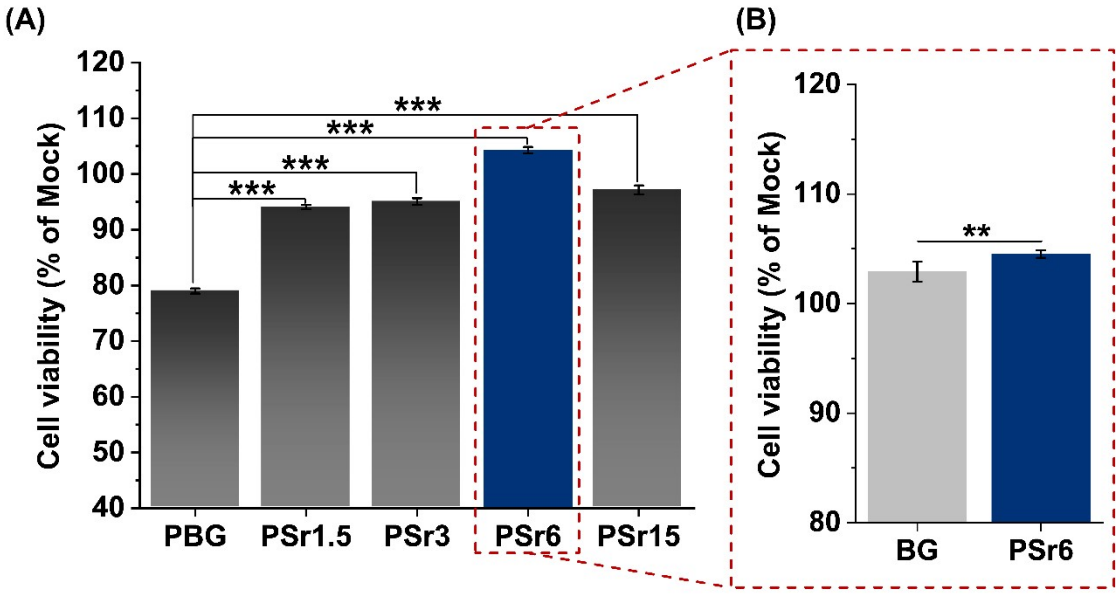


Fig. S6 Cell viability of murine macrophage cells. (A) Cell viability of PBG and PSr group results are presented as a normalized percentage of control (untreated cell culture). (B) Cell viability of BG and PSr6 ($n = 6$; ** $p < 0.01$; *** $p < 0.001$). Mock; untreated cell culture, BG; PBG; phosphate-based glass, PSr; Strontium-substituted PBG.

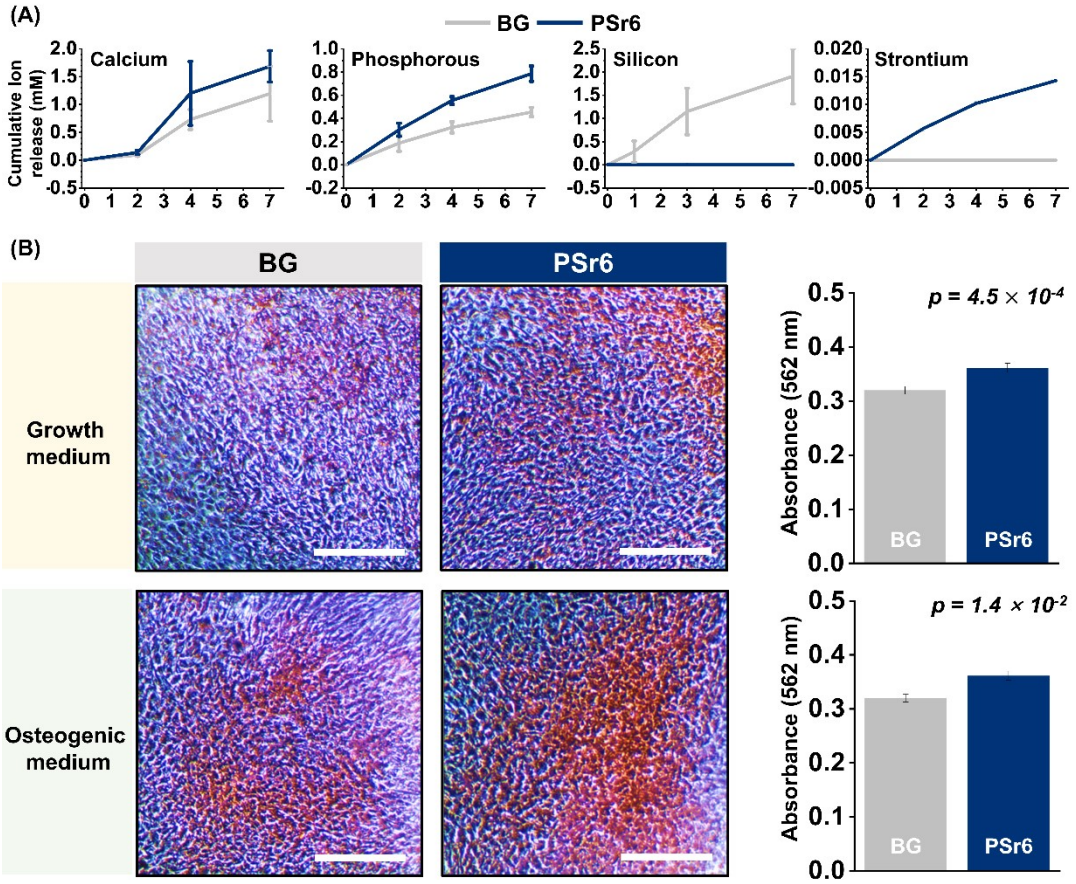


Fig. S7 (A) Cumulative ion release profile of BG and PSr6 assessed from 7 days extract in osteogenic media, corresponding to Fig.6. (B) Biomineralization assay of PSr6 as compared with benchmark BG in both growth and osteogenic medium. Observation of mineralization (red color) (Scale bar is 100 μm) and absorbance of PSr6 as compared with benchmark BG (n = 3). BG: 45S5 bioactive glass, PSr6: 6 mol% strontium-substituted phosphate-based glass.