Supporting Information

Ions release behavior of vanadium-doped mesoporous bioactive glass particles and effect on BMSCs osteogenic differentiation via FAK/MAPK signaling pathway

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1.1 Characterization of the collected V-MBG particles after soaking in DW and SBF

The morphology of the collected samples particles after immersing in DW and SBF was observed by a scanning electron microscope (SEM, S-4800, Hitachi, Japan). The phase composition of samples was characterized by an X-ray diffractometer (XRD, EMPYREAN, PANalytical B.V., Netherlands) equipped with Cu Kα radiation (wavelength 1.5418 Å), and patterns were collected in the 2θ range between 10-80° with a scan speed 5°/min. Mesoporous structures of samples also were evaluated by a transmission electron microscopy (TEM) in a microscope (Tecnai G2 F20 S-TWIN, FEI, USA). N_2 adsorption–desorption isotherms of soaked samples were acquired by a porosimeter (Kubo X1000, BEIJING Builder Electronic Technology CO., LTD, China) to determine the surface area, the pore size distribution, and the pore volume. Before the adsorption measurements, the samples were degassed under vacuum for 2 h at 200 ℃. The surface area was obtained by applying the Brunauer–Emmett–Teller (BET) method, and the pore size distribution was calculated by the Barrett–Joyner–Halenda (BJH) model from the adsorption branch of the isotherm. IR spectra of the immersed samples were recorded on the FTIR spectrometer (Nicolet 6700, Thermo Fisher Scientific, USA) in the range of 4000 to 400 cm^{-1}.

1.2 Preparation of V-MBG/PGA-PCL composite porous scaffolds

The V-MBG/PGA-PCL composite scaffolds were prepared by a solvent casting-particulate leaching method with some modification using NaCl particles as the porogen. The detailed chemical compositions and ratio were listed in Table S6. The ratio of the PGA-PCL to hexafluoroisopropanol was fixed at 10% (W/V). 1g PGA-PCL were dispersed in 10ml hexafluoroisopropanol and continuously stirred until PGA-PCL completely dissolution. And then a certain amount of V-MBG powders was added into the solution and continuously stirred for 12h and sonicated for 30min to disperse the glass powders uniformly. The mass ratio of porogen to PGA-PCL was 6:1. Sodium chloride (NaCl) particles with diameters 300-450μm were then incorporated into the suspension, and continuously stirred for 15min. Next, the suspension solution was dropped slowly into the solvent of ethanol while stirring. Then, the V-MBG/PGA-PCL precipitates were continuously washed in ethanol under stirring for 3 hours. The V-MBG/PGA-PCL precipitates were collected and then loaded into a stainless steel mold with 6mm diameter and pressed at 10 MPa for 5 min under room temperature to obtain a cylindrical specimen of about 15mm height. The specimens were then immersed in double deionized water for 72h to leach NaCl and finally dried in the room temperature.

1.3 Implant procedures of V-MBG/PGA-PCL scaffolds for repairing calvarial bone defects

The preparation of V-MBG/PGA-PCL composite scaffolds could refer to supporting information 1.2. Four-week-old male CD 57 rats were obtained from Chengdu Dossy Experimental Animals CO., LTD. After adaptation for one weeks, 14~20 g CD 57 rats were used for establishing the skull bone defect model. Briefly, the mice were anesthetized with a 1% sodium pentobarbital (50 mg/kg body weight). First, the head skin to be incised was sterilized with 70% ethanol and Betadine and incised with surgical scissors. Two defects were made on the left and right sides of the skull by using a 4-mm-diameter micro bone drill. The grilled holes were rinsed by injection with saline solution in order to remove bone fragments from the cavity, then, 4-mm-diameter MBG/PGA-PCL scaffolds (Ø4×2mm) were implanted into the defect sites. The skin was then closed using sutures. Thereafter, the rats were divided into five groups: (i) no treatment (control), n=6; (ii) PGC/V-M0 scaffolds, n=6; (iii) PGC/V-M10 scaffolds, n=6; (iv) PGC/V-M25 scaffolds, n=6, and (v) PGC/V-M40 scaffolds, n=6.

1.4 Micro-CT analysis

In order to evaluate the in vivo bone ingrowth of the implanted porous scaffolds, the calvarial bones were harvested 8 weeks after implantation. All samples for micro-CT analysis were fixed with 4% paraformaldehyde for 24 h at room temperature. Bone formation in the defective sites was visualized by a high-resolution Micro-CT Systems (VivaCT 80, SCANCO Medical AG, Switzerland). Scanning was performed at 70 kV and 114μA with a thickness of 0.015 mm per slice in medium-resolution mode, and 200 ms integration time. VG Studio software (Volume Graphics, Germany) was served for the visualization of the reconstructed 3D images.

1.5 Histological analysis

The skulls of week 8 time point were fixed with 4% paraformaldehyde at room temperature for 24h, and subsequently decalcified in 10% EDTA for 4 weeks. Then, the samples were dehydrated in grade ethanol, embedded in paraffin. The embedded specimens were cut into 5μm thick sections using a microtome (RM 2016, Leica, Germany). Hematoxylin and eosin (H&E) staining, Masson's Trichrome staining were performed according to manufacturer's protocol for observation of new bone formation.

Table S1 Chemical composition and the amounts of reactants of V-MBG

Sample	$Na_3VO_4 \cdot 12H_2O$ g	P123 g	TEOS g	TEP g	$Ca(NO_3)_2 \cdot 4H_2O$ g	HCl ml	Composition (mol, %)
MBG	0	1	2.833	0.3301	1.98	50	57.2 SiO_2 - 35.3 CaO - 3.75 P_2O_5 – 0 V_2O_5.
1.0V-MBG	0.068	1	2.833	0.3301	1.98	50	56.8 SiO_2 - 35.0 CaO - 3.75 P_2O_5 - 0.35 V_2O_5
4.0V-MBG	0.2721	1	2.833	0.3301	1.98	50	55.6 SiO_2 - 34.3 CaO - 3.70 P_2O_5 – 1.39 V_2O_5
10.0V-MBG	0.68	1	2.833	0.3301	1.98	50	53.4 SiO_2 - 32.9 CaO - 3.55 P_2O_5 - 3.34 V_2O_5

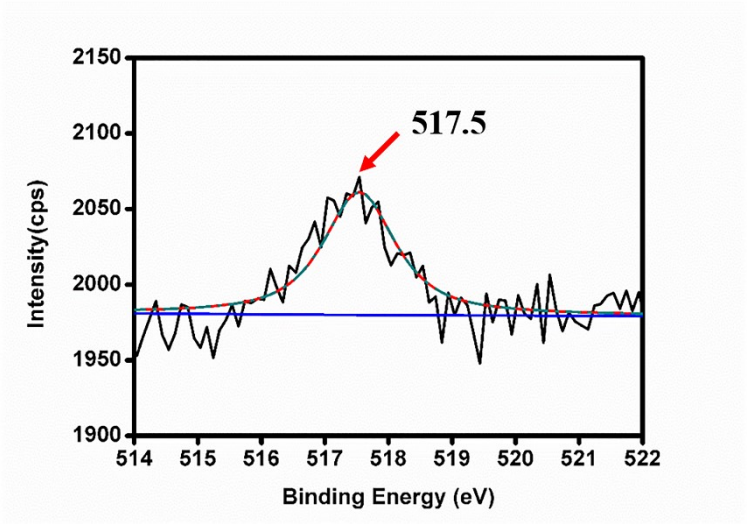

Fig. S1 Vanadium of XPS spectrum of 1.0V-MBG particle.

Table S2. Mass percent of different elements in MBG and MBG-V powers

	Mass percent / %			
	MBG	1.0V-MBG	4.0V-MBG	10.0V-MBG
Si	37.7	40.5	39.9	38.9
Ca	3.9	3.4	3.9	4.4
P	0.76	0.74	0.83	0.88
V	---	0.12	0.47	1.30
Ca/P	5.13	4.59	4.70	5.00

Table S3. Primer sequences used in Real time-PCR.

Target gene	Primers (5’–3’; F = forward; R = reverse)
Itga 2b	F: CAGACACATCTGCTTCGGGC
	R: TGAAGAAGCCAGCCTTCCACAT
FAK	F: ATACACCATGCCCTCAACCAGG
	R: TGCAACAGCCAAAGCTGGATT
ERK1	F: GCCCATTGCTGAAGCACCAT
	R: GGAGTTCATCTCTAGCACTGACCA
P38	F: CCAGCTTCAGCAGATAATGCGT
	R: TTCTTGCCTCATGGCTTGGC
JNK	F: ATGGCTCTCAGCATCCGGTC
	R: GCTGTCTGTATCCGAGGCCA
BMP-2	F: GGGACCCGCTGTCTTCTAGT
	R: CATGCTGAGCAGCCTCAACT
COLI	F: CGAGTCACACCGGAACTTGG
	R: CCAATGTCCAAGGGAGCCAC
GAPDH	F: CCCCCAATGTATCCGTTGTG
	R: TAGCCCAGGATGCCCTTTAGT

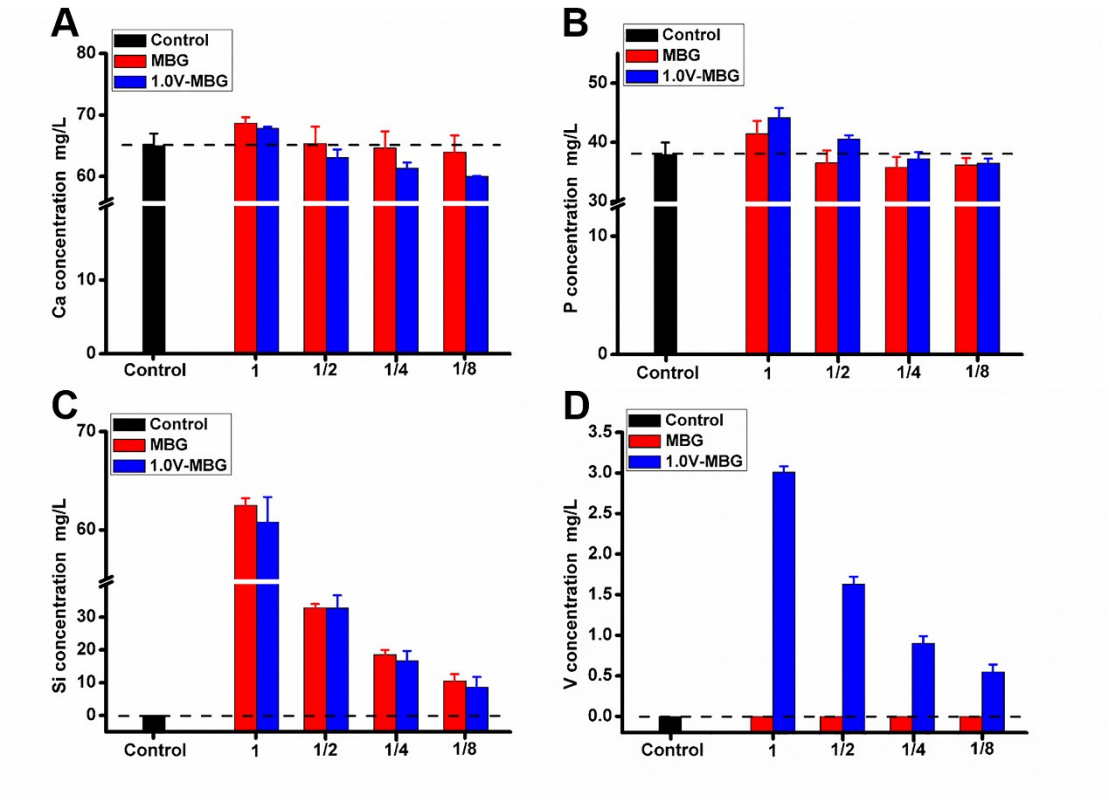

Fig. S2 Ca (A), P (B), Si (C), and V (D) ion concentrations in diluted extracts after soaking mesoporous MBG and 1.0V-MBG samples for 24h.

Table S4 Structural parameters of different samples after immersed in the distilled water for 14days

Sample	Surface area S_{BET} (m^2 g^{-1})	Pore diameter D_p (nm)	Pore volume V_p (cm^3 g^{-1})	Micropore volume V_m (cm^3 g^{-1})
MBG	254	6.58	0.647	0.107
1.0V-MBG	264	6.57	0.634	0.107
4.0V-MBG	270	6.43	0.610	0.112
10.0V-MBG	236	6.61	0.547	0.099

Table S5 Structural parameters of different samples after immersed in the SBF for 14days

Sample	Surface area S_{BET} (m^2 g^{-1})	Pore diameter D_p (nm)	Pore volume V_p (cm^3 g^{-1})	Micropore volume V_m (cm^3 g^{-1})
MBG	263	6.60	0.702	0.107
1.0V-MBG	259	6.72	0.643	0.112
4.0V-MBG	269	6.48	0.596	0.110
10.0V-MBG	156	6.25	0.469	0.046

The results of Table S4 and S5 showed that S_{BET} of the V-MBG after soaked in DW and SBF exhibited a significant decrease while pore size and pore volume displayed insignificant variation compared to the structural parameters of corresponding unsoaked sample (Microporous and Mesoporous Materials, 2021, 319, doi.org/10.1016), demonstrating that the HCA mainly formed in the surface of V-MBG.

Table S6 Component composition of V-MBG/PGA-PCL scaffolds

Group	PGA-PCL/g	V-MBG/g	V-MBG/%	MBG and 10.0V-MBG mixture	
				MBG	10.0V-MBG
PGC	1	0	0	0	0
PGC/V-M0	1	0.68	40	40	0
PGC/V-M15	1	0.68	40	25	15
PGC/V-M25	1	0.68	40	15	25
PGC/V-M40	1	0.68	40	0	40