Science Advances
AAAS

Supplementary Materials for

A biopolymer hydrogel electrostatically reinforced by amino-functionalized bioactive glass for accelerated bone regeneration

Xinxin Ding, Junyu Shi, Jianxu Wei, Yuan Li, Xiangbing Wu, Yi Zhang, Xue Jiang, Xiaomeng Zhang*, Hongchang Lai*

*Corresponding author. Email: hongchanglai@126.com (H.L.); zhangxiaomengwowo@126.com (X.Z.)

Published 10 December 2021, *Sci. Adv.* **7**, eabj7857 (2021)
DOI: 10.1126/sciadv.abj7857

This PDF file includes:

Supplementary Methods
Figs. S1 to S4
Tables S1 and S2

Methods

Si release in the cell culture medium

To identify the amount of silicon ions (Si) released from hydrogels *in vitro*, samples (diameter 8mm, thickness 1 mm) were immersed in 5 mL of cell culture medium at 37°C. After 1, 3, 7, 14 days, the medium was collected and transferred to new tubes. The Si concentration in the medium was determined using inductively coupled plasma atomic emission spectrometry (ICP-AES, iCAP6300, Thermo, USA).

Fabrication of hydrogels

AG was prepared by dissolving alginate (3% w/v, Sigma – Aldrich) and gellan gum (3.5% w/v, Sigma – Aldrich) in a 0.03% w/v $CaCl_2$ solution (Aladdin, China) followed by magnetic stirring at 90 °C and 300 rpm for 1 h. The solution was cooled to room temperature and further placed in 1M $CaCl_2$ for 10 min to enhance hydrogel crosslinking. The matrices containing BG at a concentration of 0.1% w/v, 1% w/v, 3% w/v and 6% w/v were prepared according to the same method described above. AG, AG-0.1BG, AG-1BG, AG-3BG and AG-6BG denote the hydrogels including different concentration of BG (0%, 0.1%, 1%, 3% 6%) respectively.

Cell culture

The cells used in the present study were rat bone marrow stem cells (BMSCs, passage 3-5). They were cultured in Dulbecco's modified Eagle's medium (DMEM, Hyclone, USA) supplemented with 10% heat-inactivated fetal bovine

serum (Hyclone, USA) at 37°C in a humidified CO_2 incubator. Hydrogels were prepared with punches and sterilized by ultraviolet light for 1 h before use.

Cell viability

Cell viability was detected using a CCK-8 kit (Beyotime, China). A seeding density of 5000 cells per well in a 96-well tissue plate was used. After 24 hours, CCK-8 solution was added to the culture plate and further incubated for 1 h at 37 °C. The optical density (OD) of the mixture was measured using a Synergy HT spectrophotometer (BioTek Instruments).

Alkaline phosphatase (ALP) activity

The experiment was conducted following the protocol of an ALP kit (Beyotime, China). Briefly, BMSCs were seeded at a density of 10^5 cells/mL on hydrogels. After incubation for 7 days, cells were lysed and reacted with chromogenic agents. The optical density (OD) value at 405 nm was recorded. The final alkaline phosphatase (ALP) activity was expressed by the DEA activity unit which is calculated using a formula in the kit.

Figures

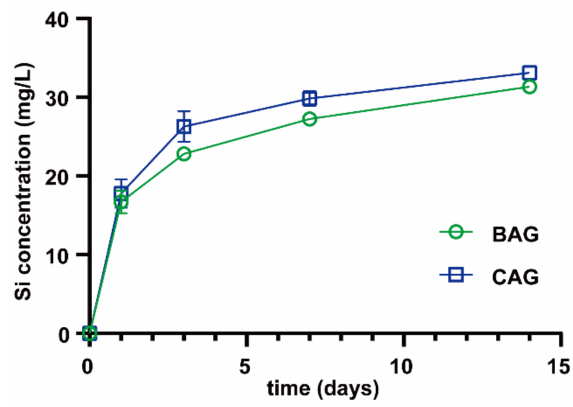

Figure S1. The concentration of Si released from hydrogels at 1, 3, 7, 14 days.

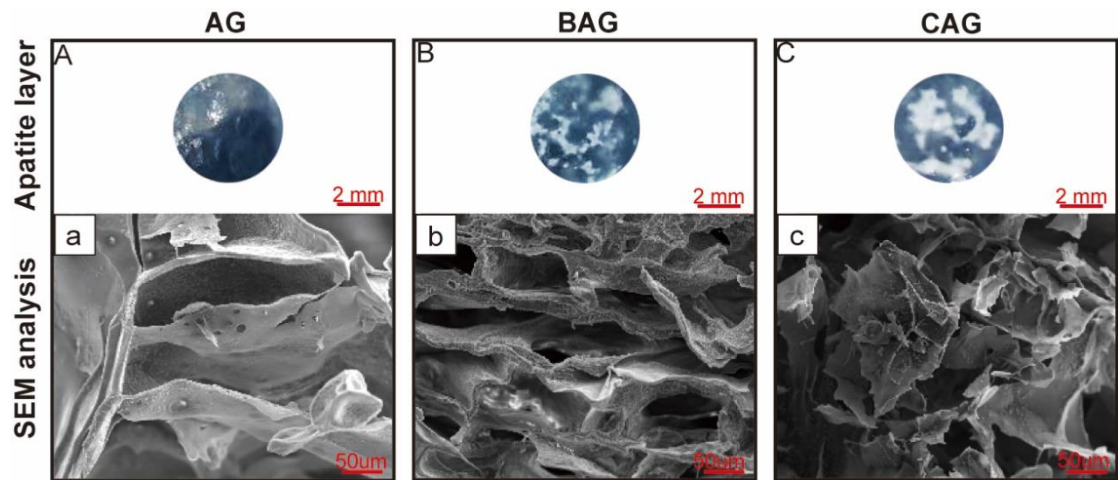

Figure S2. The formed apatite layer after hydrogels immersing in SBF solution for 28 days. **(A-C)** Images of mineralized hydrogels. **(a-c)** SEM analysis of mineralized hydrogels.

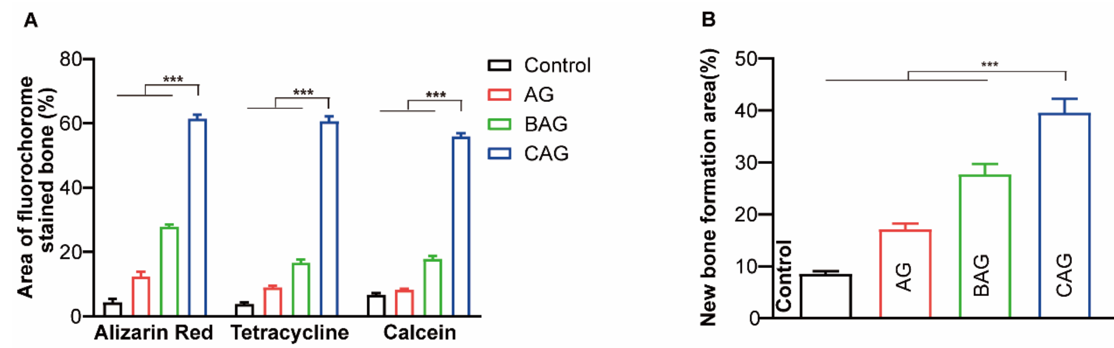

Figure S3. Quantitative analysis of area for fluorochrome stained bone and HE-stained new bone *in vivo*. **(A)** Quantitative analysis of area for fluorochrome stained bone. **(B)** Quantitative analysis of the newly bone formation at 8 weeks from HE staining images. *P < 0.05.

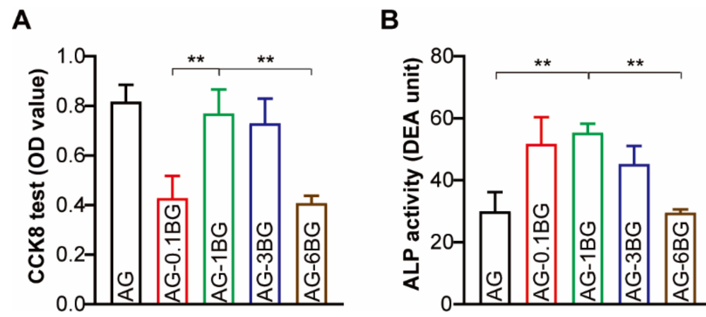

Figure S4. The biocompatibility of cells cultured on hydrogels with different concentration of BG. **(A)** CCK-8 assay of cells cultured for 24 h. **(B)** ALP activity of cells cultured for 7 days. *P < 0.05.

Table S1. PCR primer pairs for macrophage.

Gene	Forward sequence	Reverse sequence
TNF-α	CAGGCGGTGCCTATGTCTC	CGATCACCCCGAAGTTCAGTAG
IL-1	CTTCAGGCAGGCAGTATCACTC	TGCAGTTGTCTAATGGGAACGT
IL-6	ACAACCACGGCCTTCCCTAC	TCTCATTTCCACGATTTCCCAG
IL-10	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
GAPDH	ATGGGTGTGAACCACGAGA	CAGGGATGATGTTCTGGGCA

Table S2. PCR primer pairs for BMSCs.

Gene	Forward sequence	Reverse sequence
RUNX2	TCCGCCACCACTCACTACCAC	GGAACTGATAGGACGCTGACGAAG
ALP	GCCTACTTGTGTGGCGTGAA	AGGATGGACGTGACCTCGTT
OCN	GGACCCTCTCTCTGCTCACTCTG	ACCTTACTGCCCTCCTGCTTGG
COL-1	CGAGTCACACCGGAACTTGG	CCAATGTCCAAGGGAGCCAC
β-actin	CATCCGTAAAGACCTCTATGCCAAC	ATGGAGCCACCGATCCACA