# Supplementary material

## S1 Characterization of the Van-PEM hydrogel

Hematoxylin and eosin (H&E) and 406-diamino-2-phenylindole (DAPI) staining were used to determine the effect of decellularization. According to the instructions of the kit, the DNA in the periosteum was extracted in solution, and the content of DNA was calculated by the absorbance intensity of the microplate reader at 260 nm. The microstructures of the PEM and Van-PEM hydrogels were observed using scanning electron microscopy (SEM). In brief, after the hydrogel was fixed in 2.5% (w/v) glutaraldehyde for several hours, the sample was cleaned with PBS solution three times (each cleaning period was 10 min) and washed with PBS for 5 min three times. The samples were then dehydrated with 30, 40, 50, 70, 80, 90, 95, and 100% $CH_3CH_2OH$ sequentially (10 min at each gradient). A critical-point dryer was used to dehydrate the samples and sputtered the samples with Au–Pd. The structural characteristics of the PEM and Van-PEM hydrogels were investigated by SEM (JSM-6360LV, JEOL, Tokyo, Japan), and their composition was determined by energy-dispersive X-ray spectroscopy (EDS). Fourier transform infrared (FTIR) spectra of the PEM and Van-PEM hydrogels were also recorded.

## S2 Cytotoxicity tests

The same volume of PEM and Van-PEM hydrogels were added to the cell culture dish and sterilized by irradiation. Bone marrow mesenchymal stem cells (MSCs) were seeded at a density of $4 \times 10^4$ cells/well in blank wells, PEM hydrogel, and Van-PEM hydrogel. An appropriate amount of Dulbecco's Modified Eagle Medium (DMEM) medium was added to the Petri dishes, and then the Petri dishes were placed in a 37°C incubator containing 5% carbon dioxide. After 48 h, the culture medium was discarded, and the Petri dish was washed twice with PBS solution. The cells were stained with calcein reagent for 30 minutes, and then the number of living cells was observed under the fluorescence microscope.

The cytotoxicity of the PEM and Van-PEM hydrogels was evaluated using CCK-8. The PEM and Van-PEM hydrogels were immersed in the medium, and the volume ratio of hydrogel to medium was 1:5. After two days, the culture medium was sucked out as the leaching solution, which was then mixed with the culture medium in a certain proportion to prepare the culture medium containing 0, 20, 50, 100% leaching solution. In 96 well cell culture dishes, 2000 cells were added to each well, and an appropriate amount of DMEM medium was added for incubation for

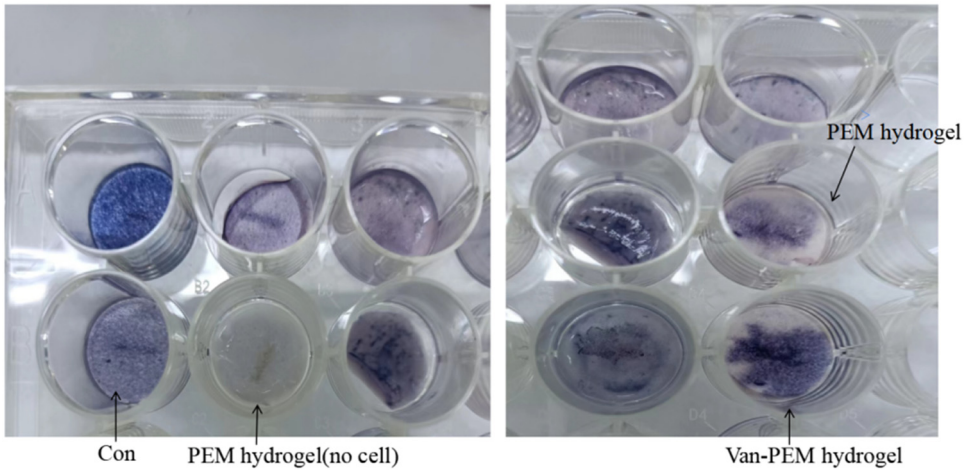


**Figure S1:** General view of cell culture cluster for ALP staining of osteoblasts cultured in the PEM and Van-PEM hydrogels.

**Table S1:** Primers used for the quantitative real-time reverse transcriptase polymerase chain reaction (RT-qPCR)

| Gene target | Sequence |
|---|---|
| GAPDH | Forward: 5′-GAGACCTTCAACACCCCAGC-3′ |
| | Reverse: 5′-ATGTCACGCACGATTTCCC-3′ |
| RUNX2 | Forward: 5′-CGCCTCACAAACAACCACAG-3′ |
| | Reverse: 5′-TGCTTGCAGCCTTAAATATTCCTG-3′ |
| OSX | Forward: 5′-GCCCACTGGTGCCCAAGACC-3′ |
| | Reverse: 5′-CCCGTGGGTGCGCTGATGTT-3′ |
| OCN | Forward: 5′-CTGACCTCACAGATCCCAAGC-3′ |
| | Reverse: 5′-TGGTCTGATAGCTCGTCACAAG-3′ |

24 h. Then, the supernatant was discarded and the medium containing 20, 50, and 100% leaching solution was added for culture. On the first, third and fifth days, we added reagents to the Petri dish according to the instructions of CCK-8 kit and detected the absorbance of cells by microplate reader to determine the growth of cells.

## S3 Statistical analysis

All data are expressed as the mean ± standard deviation, and Graphpad Prism software (version 8.0) was used for statistical processing. One-way analysis of variance (ANOVA) and unpaired *T*-tests were used to determine whether there were significant differences. Statistical significance was set at $p < 0.05$.