Supporting Information

Biomimetic Mineralizable Collagen Hydrogels for Dynamic Bone Matrix Formation to Promote Osteogenesis

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1. Nuclear magnetic resonance (NMR) spectrum Analysis

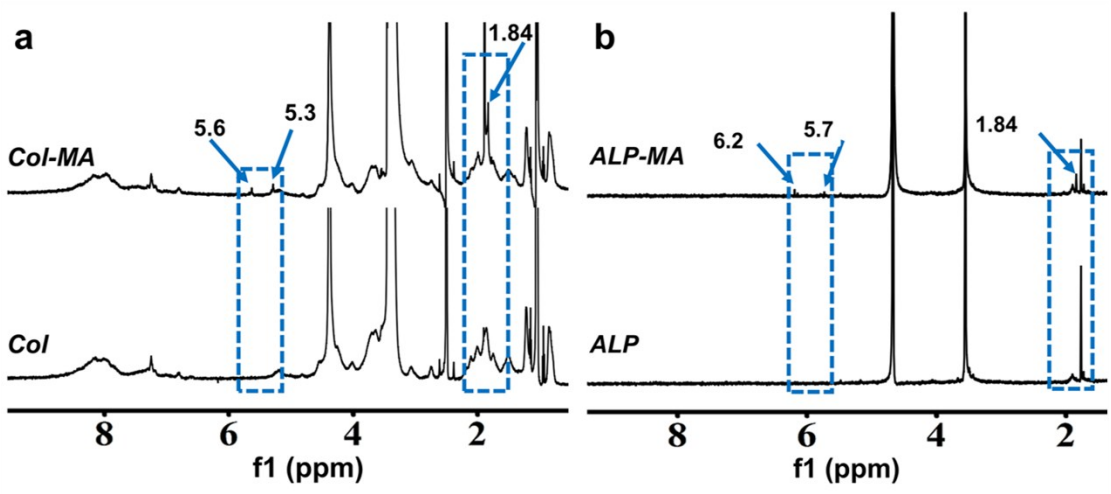


Fig. S1 NMR spectra of Col and ALP before and after metharylamide. (a) H NMR spectra of Col and Col-MA. (b) 1H NMR spectra of ALP and ALP-MA.

The modification of methacrylate groups onto Col and ALP molecules was confirmed by NMR analysis (Fig. S1). As shown in Fig. S1a, the appearance of methacrylamide proton peaks (methylene at 5.6 and 5.3ppm, methyl at 1.84 ppm) in the spectrum of Col-MA confirmed the incorporation of methylpro pylene photoactive moieties. In Fig. S1b, the presence of methylene at 6.2 and 5.7 ppm, the methyl peak at 1.84 ppm, proved the incorporation of methacrylamide photoactive moieties in ALP-MA. Therefore, through methacrylamide modification, ALP-MA and Col-MA were successfully prepared, thus biomimetic mineralizing collagen hydrogel could be constructed by photo-crosslinking ALP-MA and VAP in collagen networks.

2.Varied forms of Ca and P

Table 1 Varied forms of Ca and P

Component	SBF	CaGP
Ca	Free ion, 2.5mM	Free ion, ~9mM
P	Free ion, 1mM	Organic phosphorus, ~ 9mM

The phosphate group exists as different forms in SBF and CaGP. It exists as free ion in SBF while as organic phosphates in CaGP. Only enzymatic hydrolysis of CaGP can produce free phosphate ion, and then combine with Ca^{2+} to form calcium phosphate.

3. EDS analysis

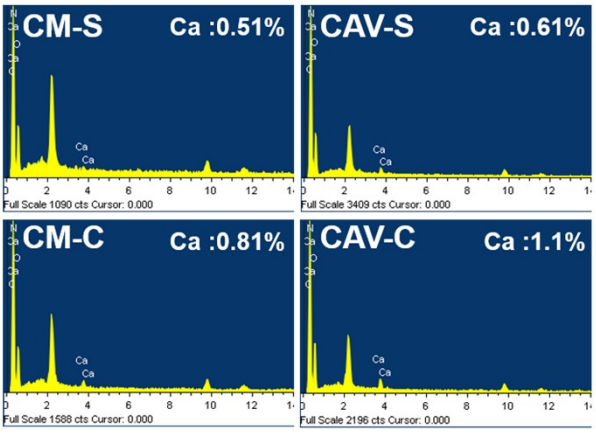


Fig. S2 EDS analysis of CM and CAV mineralization in SBF (S) or 0.2% CaGP (C) solution.

EDS analysis (Fig. S2) shows that the content of Ca in CM-C or CAV-C was higher than that of CM-S or CAV-S. However, no matter in what medium, the content of Ca was higher in CAV hydrogels than that in CM hydrogels.

4. Enzymatic hydrolysis of CAGP by alkaline phosphatase

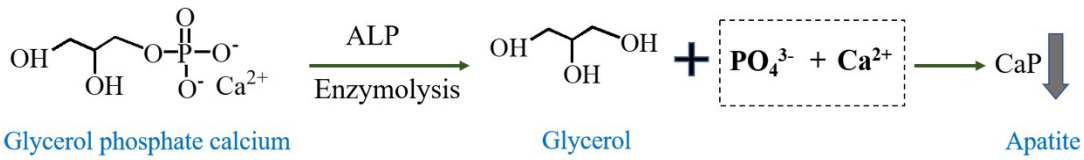


Fig. S3 The reaction of alkaline phosphatase enzymolysis CaGP to produce CaP.

5. EDS analysis of CM-M and CAV-M mineralization with cell participated

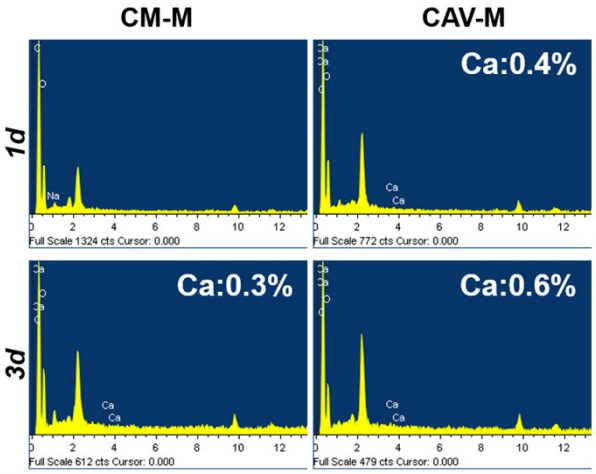


Fig. S4 EDS analysis of CM-M and CAV-M mineralization with cell participation.

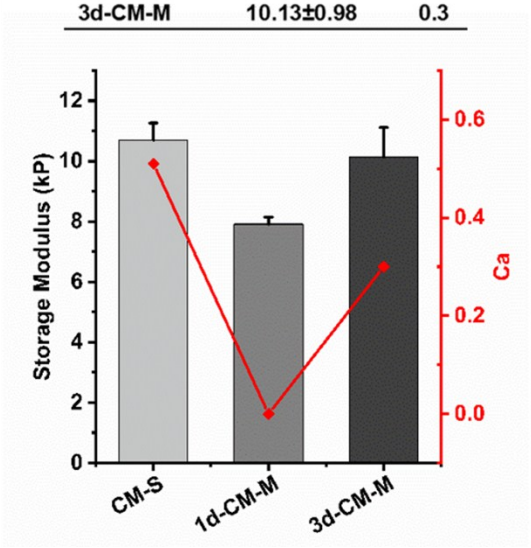
As showed in Fig. S4, EDS observation demonstrated that the deposited apatite increased gradually both in CM-M and CAV-M with time prolongation from day 1 to day 3, and CAV-M induced more mineral deposition than CM-M.

6. Relationship between gel strength as well as Ca content and cell participation in mineralization

As shown in Fig. S5, the amounts of CaP deposition in cell participated mineralization hydrogels (CM-M or CAV-M) are lower than that in SBF (CM-S or CAV-S) without cell participation, but the strength of CAV-M is higher than CAV-S. This indicates that some new matrix secreted by cells participates the dynamic matrix formation and synergistic increase the matrix strength in CAV-M.

	Storage modulus (kP)	Ca%
CM-S	10.7±0.56	0.51
1d-CM-M	7.91±0.24	--
3d-CM-M	10.13±0.98	0.3

	Storage modulus (kP)	Ca%
CAV-S	5.21±0.30	0.61
1d-CAV-M	5.64±0.11	0.4
3d-CAV-M	14.98±1.73	0.6



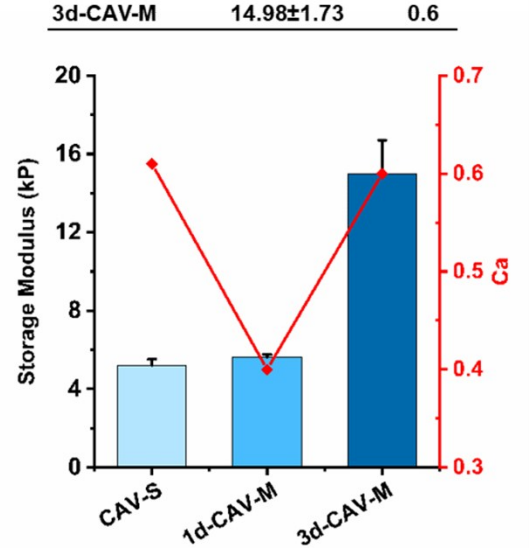


Fig. S4 Relationship between gel strength as well as Ca content and cell participation in mineralization.

References:

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2. K. Yang, J. Sun, Z. Guo, J. Yang, D. Wei, Y. Tan, L. Guo, H. Luo, H. Fan and X. Zhang, J. Mater. Chem. B, 2018, 6, 7543-7555.;

3. K. Yang, J. Sun, D. Wei, L. Yuan, J. Yang, L. Guo, H. Fan and X. Zhang, J. Mater. Chem. B, 2017, 5, 8707-8718.;3.