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Biomimetic Mineralization of Acid Polysaccharide-based Hydrogels: Towards Porous 3-Dimensional Bone-like Biocomposites

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Synthesis of Maleic Chitosan and PEG-DA macromer precursors

The synthesis of maleic chitosan was carried out using a novel approach developed in our own group. Briefly, 1.0 g methanesulfonic chitosan salts were dissolved in 100ml DMSO solvent under stirring at room temperature. Solid maleic anhydride of 6-fold in molar ratio was then added to the solution, and the reaction mixture was stirred under N_2 at 60 °C for 24 hrs. The resulting product in the solution was precipitated out by acetone, filtered, washed with copious amounts of acetone, and dried. Methanesulfonic (or tolunesulfonic) anions in the product were then removed from the chitosan salts by adding 0.1M $NaHCO_3$ solution. Finally the solution was dialyzed against deionized water (MW cut off 12,000) and lyophilized to yield 69% of the products. Degree of substitution of maleic chitosan=1.2, with N-2 and O-6 substitution 0.75 and 0.45, respectively. Molecular weight (M_n) =56,000.

PEGDA Synthesis: Briefly, 1.5 mmol of PEG (Mn=4000) was dissolved in 150 mL of benzene and heated to 45 °C with stirring until complete dissolution. After the solution was cooled to room temperature, 1.67 mL (12.0 mmol) of triethylamine, at a fourfold molar excess concentration (based on PEG diol end groups), was added to the PEG solution. Then, 0.97 mL (12.0 mmol) of acryloyl chloride, also at a fourfold molar excess concentration, was added dropwise to the PEG solution through a syringe. The mixture was stirred and heated to 80 °C for 3 h under the protection of nitrogen gas. The reaction was then stopped, and the insoluble triethylamine was removed by filtration. The PEG-DA product was then precipitated by the addition of 700 mL of cold hexane into the solution. The PEG-DA precipitate was collected using a funnel, redissolved in 20 mL of benzene, and reprecipitated by adding 700 mL of chilled hexane twice. The PEG-DA polymer was finally dried for 24 h in a vacuum oven at 35 °C and the dry product was stored in the refrigerator (approximately 4 °C) for future use.

Derivative thermogravimetric analysis

Based on the TGA data, a derivative thermogravimetric (DTG) curve could be plotted. DTG analysis reveals the rate of change of weight and is particularly useful in defining the temperature of the initial onset of decomposition for each weight loss event. A typical example (a 7-day mineralized hydrogel sample) is provided below(Figure S5).

Based on the DTG curve, we can find several major events of weight loss for the 7-day mineralized including the evaporation of occluded waterat low temperature from 26 to 235°C (Event 1, free water evaporation: 26 to 100 °Cand bound water evaporation: 100 to 235 °C), the decomposition of organic components from 235 to 373°C (event 2) and burning of decomposed organic molecules from 373 to 700°C (event 3).

Therefore, determination of weight percentage of inorganic phase in mineralized hydrogel samples with different mineralization time (TGA curves were shown in Figure 5.) could be based on calculations from the DTG curves using the following formulas below. All the calculations were listed in **Table S2.**

Define X as: Weight loss percentage from organic part in mineralized sample in the temperature range between T_2~700°C).

Define Y as: Weight percentage of inorganic phase in the mineralized hydrogel composite (Y);

Define Z as: Weight percentage of remaining organic part in mineralized sample when heated to 700°C.

We have the following formulas to calculate X and Z:

$(W_{Control\ (T1)} - W_{Control\ (T2)})/(W_{Control\ (T2)} - W_{Control\ (T3)}) = (W_{mineralized\ sample\ (T1)} - W_{mineralized\ sample\ (T2)}) / X$; (Formula S1)

$(W_{Control\ (T1)} - W_{Control\ (T2)})/ (W_{mineralized\ sample\ (T1)} - W_{mineralized\ sample\ (T2)}) = 1.74\%/Z$; (Formula S2)

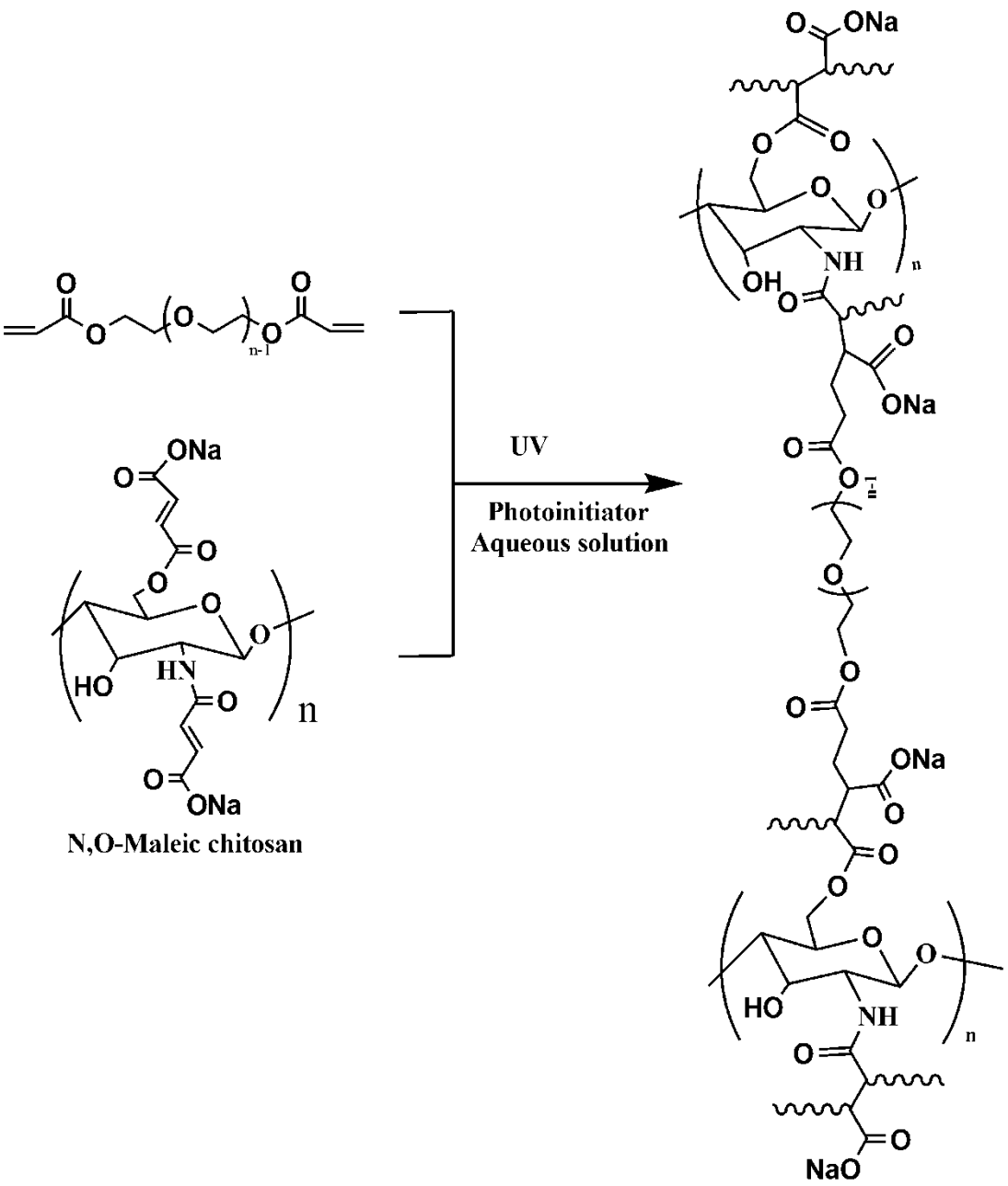
Based on the calculated X, Z values, we can calculate Y using the following formula:

$Y = (W_{Control\ (T2)} - X - Z)$ (Formula S3)

Note:

thecontrolsamplehererefersto theunmineralizedsampledriedatthesameconditions.

Dunnett's test at $P < 0.05$ was used to determine any statistical significance of the mineral amount values at different stages calculated from TGA data from samples of three batches.



Scheme S1UV-Photopolymerization between N,O-maleic chitosan and PEGDA leading to crosslinking structures

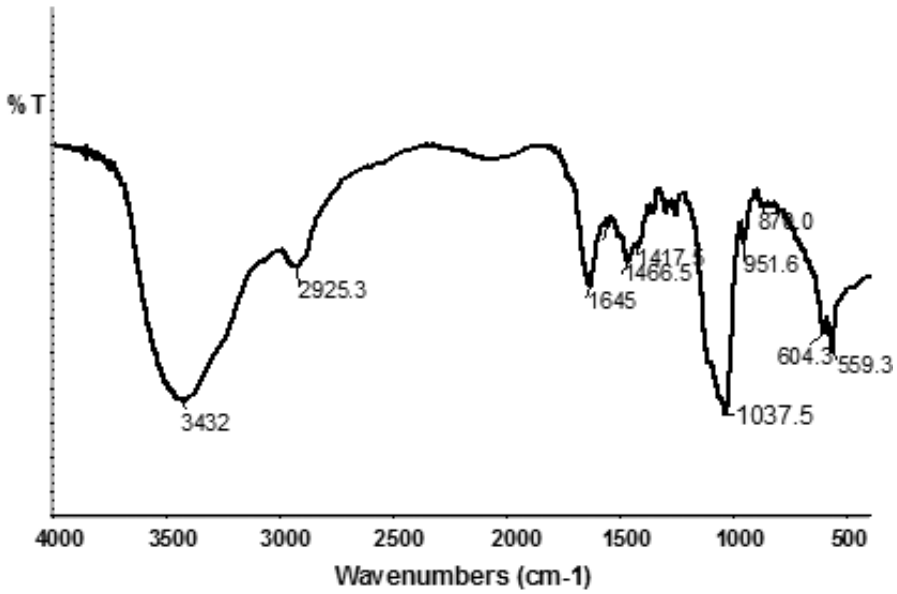


Figure S1.FTIR spectrum of 17-day mineralized hydrogel

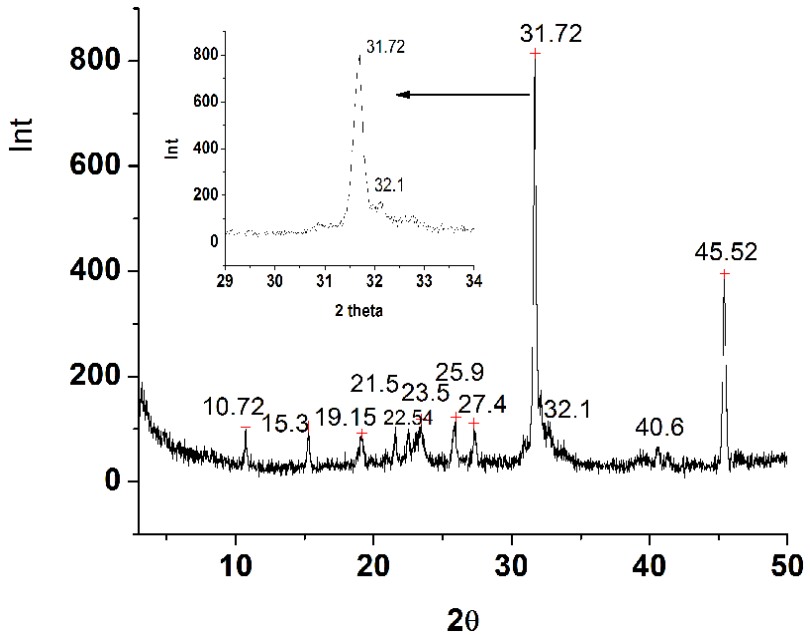


Figure S2. WXRD reflections of the CaP deposits formed on Maleic Chitosan/PEGDA hydrogels after 17-day incubation in SBF

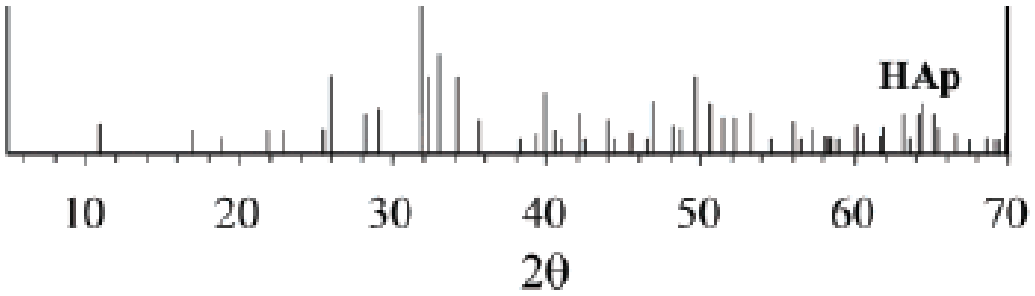
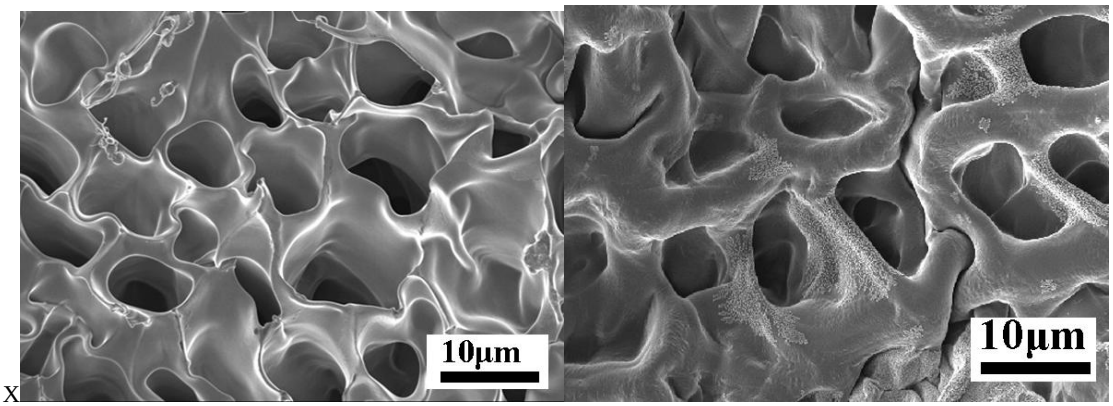
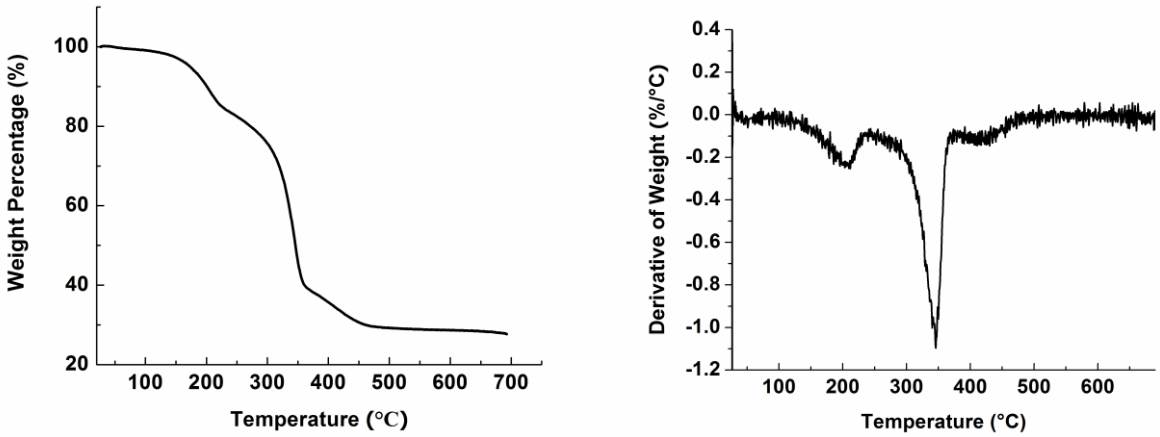


Figure S3. Standard XRD reflections of Hydroxyapatite pattern



X

Figure S4.SEM images of PEGDA hydrogel (control sample, left) and PEGDA hydrogel after immersion in SBF for 17 days (right). Mineralization was only observed to be randomly deposited in some area of the hydrogel surface.



X

Figure S5.Typical TGA and DGA of a 7-day mineralized hydrogel sample

Table S1. XRD reflections of the CaP deposits formed on maleic Chitosan/PEGDA hydrogels after 17days incubation in SBF

2θ	Reflection (hkl)	CaP phase
10.72	100	HAP
15.3		
19.15		
21.5		
22.54		
23.55		
25.80	002	HAP (Hydroxyapatite)
27.40		
31.72	211	HAP
32.1	112	HAP
39.3	212	CHAP(Carbonated HAP)
40.6	221	HAP
45.42	203	HAP

Table S2. Weight percentage of the samples at transition temperatures based on TGA curves shown in the above picture.

Samples	Control sample	Mineralized 3 days	Mineralized 7 days	Mineralized 17 days
Endset of Event1 (T_1)	220°C	240°C	240°C	240°C
Weight% at T_1	95.1%	87.1%	84.4%	84.4%
Endsetof Event2 (T_2)	390°C	385°C	373°C	369°C
Weight% at T_2	10.3%	28.0%	38.4%	49.8%
Endsetof Event2 T_3=700	700°C	700°C	700°C	700°C
Weight% at T_3	1.7%	18.5%	27.7%	39.3%
X		5.7%	5.2%	4.2%
Z		1.0%	0.94%	0.70%
Y		21.3%	32.3%	44.9%

X

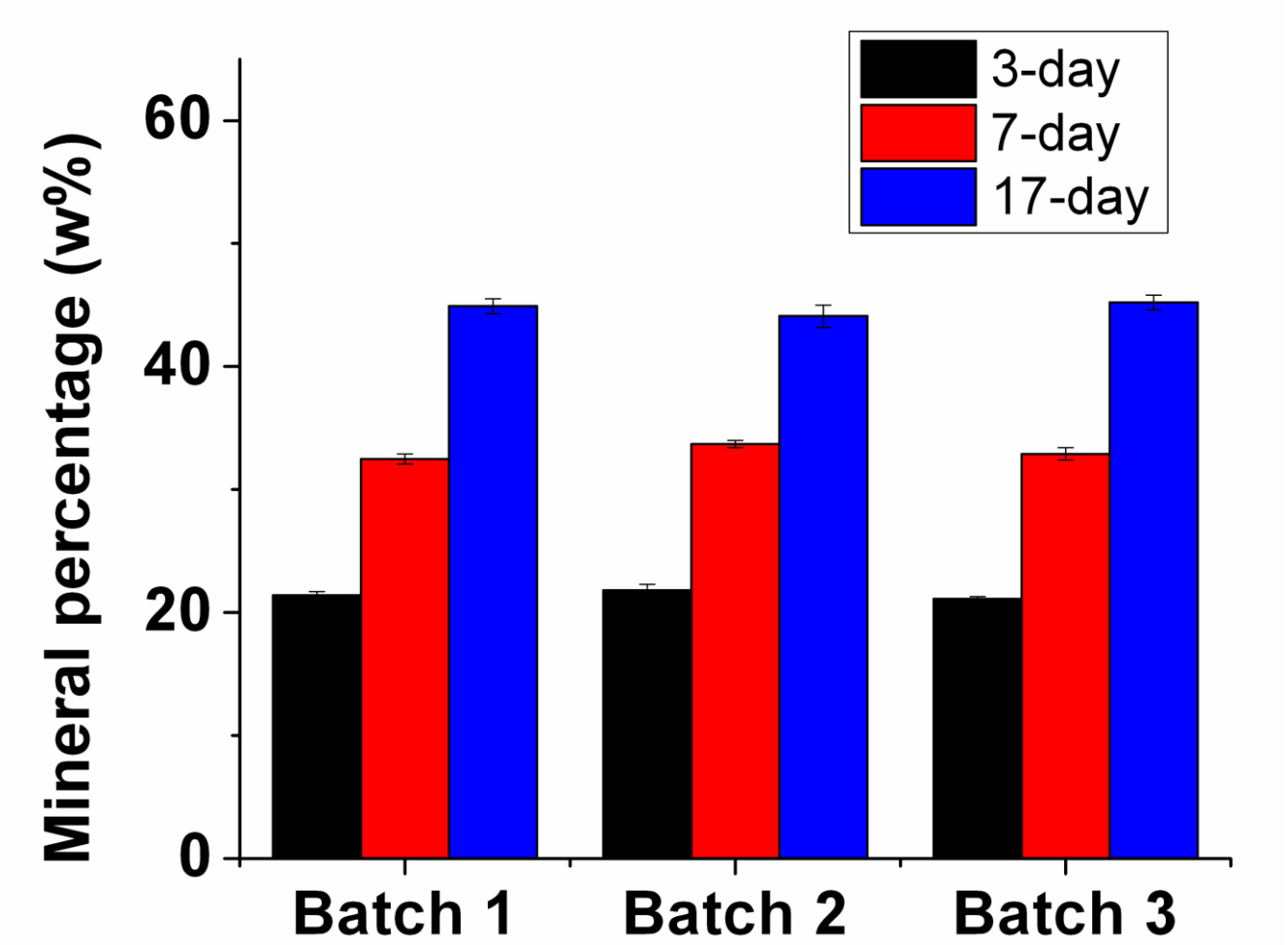


Fig.S6. Mineral percentage in the mineralized hydrogel composites at different mineralization stages carried out at the same mineralization conditions but with different batches. Dunnett's test at $P < 0.05$ was used to determine any statistical significance of the mineral amount values at different stages calculated from TGA data from samples of three batches.