

Electronic Supplementary Material**PC@PAM/CaAlg/CaCO₃ composite hydrogel films fabricated via surface mineralization and their swelling, phycocyanin release, antibacterial and antioxidant performances**

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TEXT S1: Phycocyanin (PC) standard curve

A standard curve was established by ultraviolet–visible (UV–Vis) spectrophotometry to determine the relationship between the concentration and absorbance of phycocyanin (PC). The resulting regression equation was subsequently used to quantify the PC concentration in the in vitro release experiments.

(1) Preparation of PC standard solutions

An appropriate amount of PC standard was accurately weighed and dissolved in phosphate-buffered saline (PBS, pH 7.4) to prepare a stock solution with a concentration of 0.10 mg·mL⁻¹. The stock solution was then serially diluted with PBS to obtain standard solutions with concentrations of 0, 0.01, 0.02, 0.04, 0.06, 0.08, and 0.10 mg·mL⁻¹.

(2) Absorbance measurement

PBS was used as the blank control. The absorbance of each standard solution was measured at 620 nm using a UV–Vis spectrophotometer at room temperature, corresponding to the characteristic absorption wavelength of PC. Each concentration was measured in triplicate, and the average absorbance value was used for subsequent analysis.

(3) Construction of the standard curve

The standard curve was constructed by plotting the absorbance at 620 nm (*A*) against the PC concentration (*C*, mg·mL⁻¹). Linear regression analysis was performed to obtain the regression equation and the coefficient of determination (*R*²). The standard curve was fitted according to Eq. (S1) as follows:

$$y = kx + b \quad (\text{S1})$$

where *y* is the absorbance at 620 nm, *x* is the PC concentration (mg·mL⁻¹), *k* is the slope of the regression line, and *b* is the intercept.

TEXT S2: In vitro antibacterial assay

The antibacterial activity of the hydrogel film was evaluated against *Staphylococcus aureus* and

Escherichia coli using the plate counting method. A control group and a PC@PAM/CaAlg/CaCO₃ composite hydrogel film group were included in the experiment. Bacterial suspensions were cultured in nutrient broth and diluted to a final concentration of 1×10^6 CFU·mL⁻¹. The diluted suspensions were then incubated with the hydrogel samples for 24 h. After incubation, the bacterial suspensions were serially diluted in tenfold steps, spread onto agar plates, and further cultured for colony formation. All experiments were performed in triplicate, and the results are presented as mean $\pm$ standard deviation (SD). Statistical analysis was performed using independent sample *t*-tests, with $p < 0.05$ considered statistically significant. The number of colony-forming units (CFUs) was counted, and the bacterial relative viability (η) was calculated according to Eq. (S2) as follows:

$$\eta/\% = \frac{\text{CFU}_{\text{hg}}}{\text{CFU}_{\text{cg}}} \times 100 \quad (\text{S2})$$

where CFU_{hg} is the number of colonies in the PC@PAM/CaAlg/CaCO₃ hydrogel group, and CFU_{cg} is the number of colonies in the control group.

TEXT S3 Digital photographs of the film before and after PC release

Digital photographs were recorded before and after the *in vitro* release experiment to visually monitor the release behavior of PC from the PC@PAM/CaAlg/CaCO₃ composite hydrogel film. As shown in Fig. S2, the PC-loaded film exhibited a blue color before the release experiment. After release in PBS (pH 7.4), the film became noticeably lighter in color, indicating that part of the encapsulated PC had diffused from the hydrogel network into the release medium. This visual observation is consistent with the sustained release behavior presented in the main text. It should be noted that the digital photographs are provided only for qualitative observation of the color and macroscopic appearance of the film, whereas the released amount of PC was quantitatively determined by UV–Vis spectrophotometry.

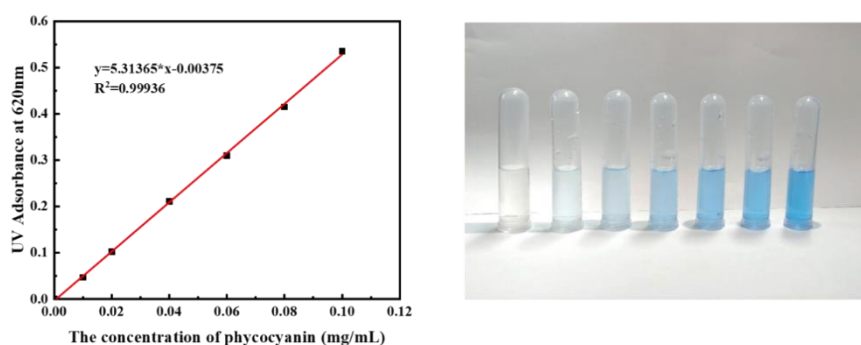


Fig. S1 Left panel: standard curve of PC in PBS. Right panel: digital photographs of PC standard solutions at concentrations of 0, 0.01, 0.02, 0.04, 0.06, 0.08, and 0.10 mg·mL⁻¹ from left to right.

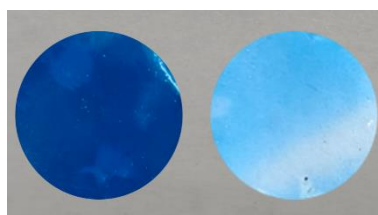


Fig. S2 Digital photographs of the PC-loaded film: before release (left panel) and after release (right panel) in PBS at pH 7.4 for 5000 min.

Table S1 PC loading capacity and encapsulation efficiency of non-mineralized and CaCO₃-mineralized hydrogel films (AM/NaAlg = 8:1)

Sample	Loading capacity/(mg·g ⁻¹)	Encapsulation efficiency/%
Non-mineralized film	2.789 ± 0.013	37.314 ± 0.35
CaCO ₃ -mineralized film	2.785 ± 0.008	37.311 ± 0.41

Table S2 Fitting parameters for the PC-release kinetics of PC@PAM/CaAlg/CaCO₃ composite hydrogel films under different AM/NaAlg mass ratios and Na₂CO₃ concentrations

AM:NaAlg mass ratio	c/wt.%	Zero-order model		First-order model		Higuchi model		Korsmeyer–Peppas model		
		<i>R</i> ²	<i>K</i>	<i>R</i> ²	<i>K</i>	<i>R</i> ²	<i>K</i>	<i>R</i> ²	<i>K</i>	<i>n</i>
4:1	0.05	0.68505	0.01573	0.93062	0.01409	0.82786	1.16857	0.99056	1.13536	0.94430
	0.10	0.60544	0.01381	0.93504	0.01717	0.76917	1.04787	0.872	7.62625	0.47212
	0.20	0.59787	0.01362	0.94191	0.02037	0.74613	1.02497	0.94401	4.58436	0.64035
	0.30	0.49487	0.01174	0.96478	0.02702	0.65377	0.90281	0.99846	4.99935	0.69429
	0.50	0.65283	0.01573	0.91463	0.01465	0.78985	1.16837	0.98311	1.34467	0.87823
8:1	0.05	0.78916	0.01573	0.79801	0.01423	0.86691	1.12089	0.86163	9.71635	0.33867
	0.10	0.64393	0.01415	0.93353	0.01874	0.78944	1.05768	0.96217	5.41132	0.58183
	0.20	0.72268	0.01542	0.91078	0.01514	0.87152	1.14518	0.98662	5.29315	0.55244
	0.30	0.49631	0.01303	0.98755	0.01994	0.67105	1.01254	0.971689	4.40519	0.66227
	0.50	0.63395	0.01605	0.95188	0.01389	0.80709	1.21918	0.97323	2.23948	0.77791
12:1	0.05	0.27624	0.00996	0.94718	0.04554	0.4657	0.81798	0.92899	7.20573	0.70619
	0.10	0.66882	0.01443	0.78221	0.02041	0.79016	1.05152	0.81966	11.5890	0.35548
	0.20	0.51687	0.01231	0.77974	0.04774	0.61995	0.90039	0.88561	6.32661	0.60363
	0.30	0.59339	0.01345	0.80632	0.02574	0.74768	1.00695	0.79447	10.74896	0.43305
	0.50	0.43274	0.01235	0.92841	0.02699	0.63051	0.97028	0.86289	9.29235	0.52372

Note: *c*, concentration of Na₂CO₃.**Table S3** Fitting parameters of different kinetic models for PC release from non-mineralized and CaCO₃-mineralized PAM/CaAlg hydrogel films

Sample	Zero-order model		First-order model		Higuchi model		Korsmeyer–Peppas model		
	<i>R</i> ²	<i>K</i>	<i>R</i> ²	<i>K</i>	<i>R</i> ²	<i>K</i>	<i>R</i> ²	<i>K</i>	<i>n</i>
Non-mineralized	0.46384	0.01715	0.97167	0.00132	0.66180	1.33333	0.94863	0.08050	1.65593
CaCO ₃ -mineralized	0.72268	0.01542	0.91078	0.01514	0.87152	1.14518	0.98662	5.29315	0.55244