

Supporting Information

Fabrication of ACP-CCS-PVA composite membrane for a potential application in guided bone regeneration

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Number of figures:6

Number of tables:1

1. Supplementary Experiment Section

1. Brunauer-Emmett-Teller (BET)

The specific surface area of the samples was measured by the Brunauer-Emmett-Teller (BET) method in the condition of liquid nitrogen using a specific surface area and porosity analyzer (AUTOSORB–IQ–MP, USA). Pore volume and pore size distribution were determined by the Barrett-Joyner-Halenda (BJH) method based on nitrogen desorption data.

2. Fourier transform-infrared spectroscopy (FT-IR)

ATP-stabilized ACP nanoparticles were soaked in PBS solution (pH 7.4, 37°C) for designated times. The dried ACP nanoparticles were mixed with potassium bromide powder in a mass ratio of about 1:50 and ground under infrared light irradiation. The mixed powder was made into round flakes using a tablet machine and was analyzed by Fourier transform infrared spectroscopy (Spectrum 400, Perkin-Elmer, USA). The wavelength number of the FT-IR spectrum was set to 400-4000 cm^{-1} , the resolution was 4 cm^{-1} , and a total of 20 scans were performed.

3. Swelling Behavior

Each sample's dry weight (W_d) was measured immediately after vacuum drying (FreeZone®, Labconco, USA). The composite membranes were then immersed in 0.9% saline at 37°C until swelling equilibrium was reached and the expansion weight (W_s) was determined. The swelling ratio (SR) of each sample is calculated as follows: $SR = ((W_s - W_d) / W_d) \times 100\%$.

4. Cell viability

Fibroblast L929 cells (Cellbank of Chinese Science Academy, China) were incubated in high-glucose Dulbecco's modified Eagle medium (DMEM) (Sigma, USA) supplemented with 10% FBS (GIBCO, USA), 100 mg/ml streptomycin and 100 U/mL penicillin in a humidified atmosphere of 5% CO₂ at 37 °C. The medium was replaced every 2–3 days, and the cells were passaged when the density reached 80–90% confluence.

Cell viability was tested using a CCK-8 assay (Dojindo, Japan). The composite membranes were placed in 48-well plates, and 0.5×10^4 cells/well were seeded into each well. The O.D. value of the experimental group at 450 nm was measured with a microplate reader (PerKinElmer, USA). Cell viability was obtained using the following formula: percentage of cell viability = $(A_{\text{treatment}} - A_{\text{blank}}) / (A_{\text{control}} - A_{\text{blank}}) \times 100\%$ (where, A = absorbance).

2. Supplementary Figures

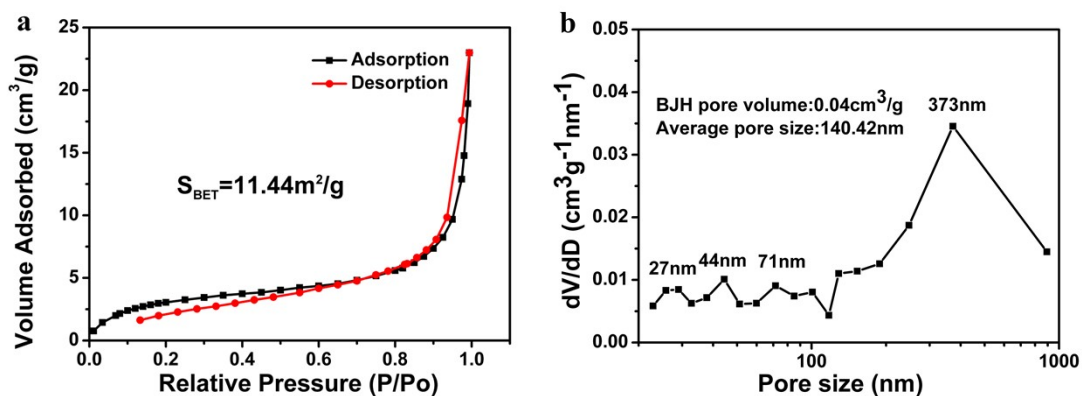


Fig. S1 (a) N₂ adsorption-desorption isotherms and (b) Barrett-Joyner-Halenda (BJH) desorption pore size distribution curve of 10 wt% ACP-CCS-PVA composite membranes.

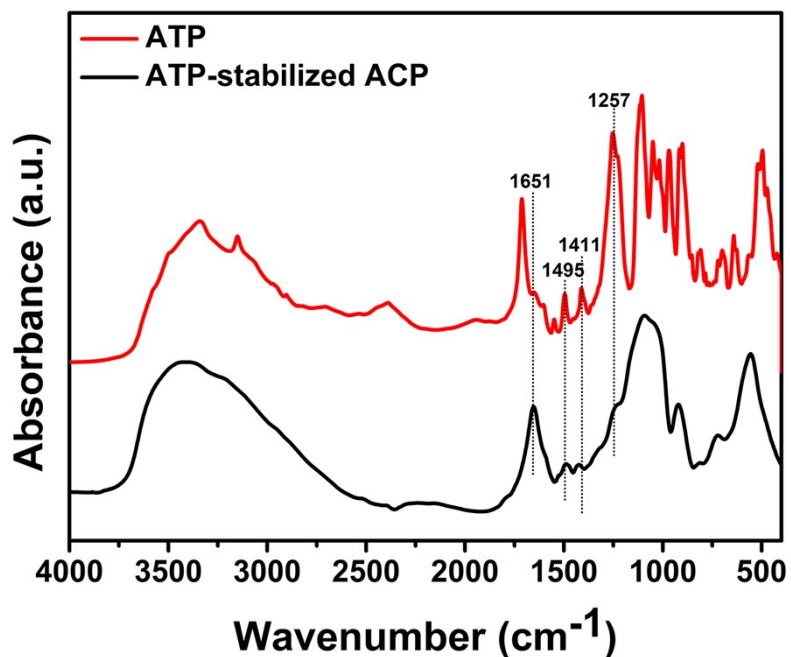


Fig. S2 FTIR spectra of the ATP-stabilized ACP nanoparticles and ATP.

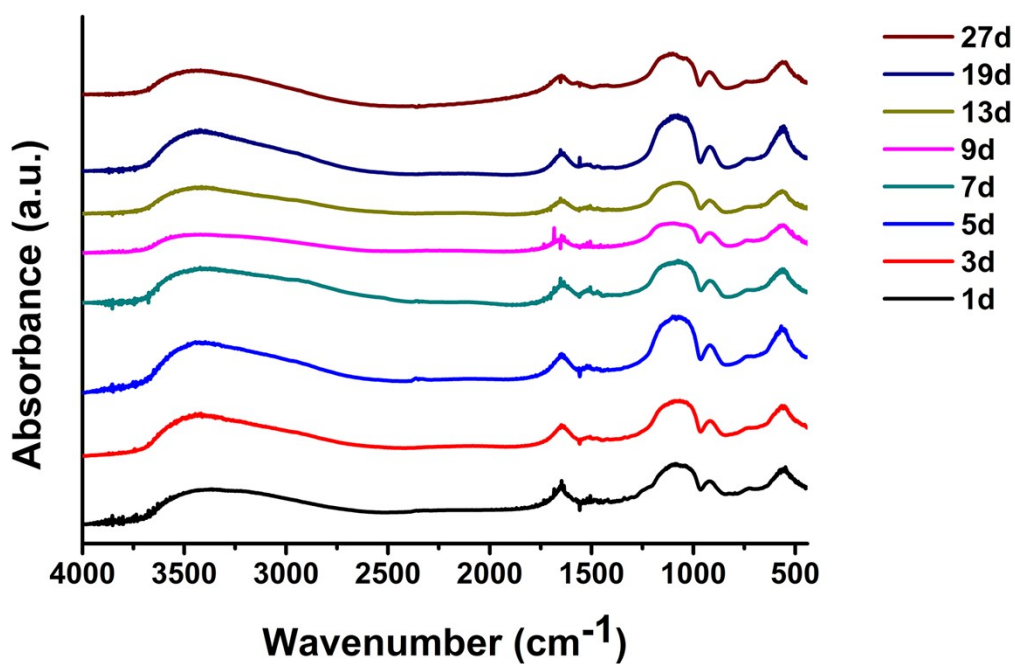


Fig. S3 FTIR spectra of the ATP-stabilized ACP nanoparticles after soaking in PBS solution (pH 7.4) for designated times.

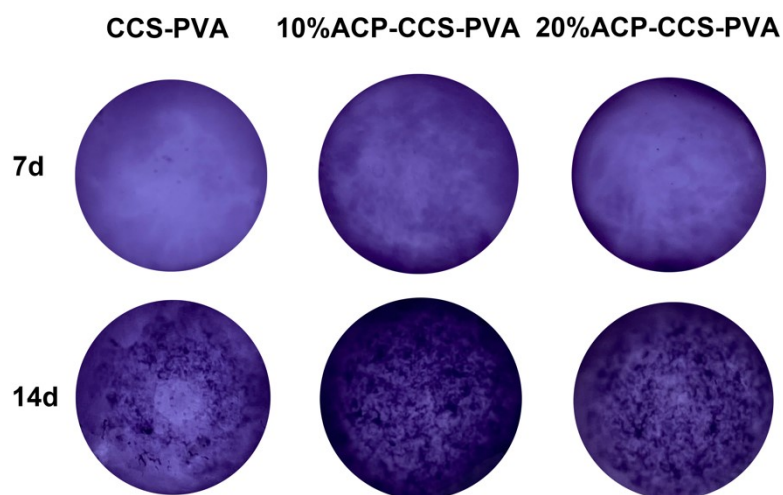


Fig. S4 FTIR spectra of the ATP-stabilized ACP nanoparticles after soaking in PBS solution (pH 7.4) for designated times.

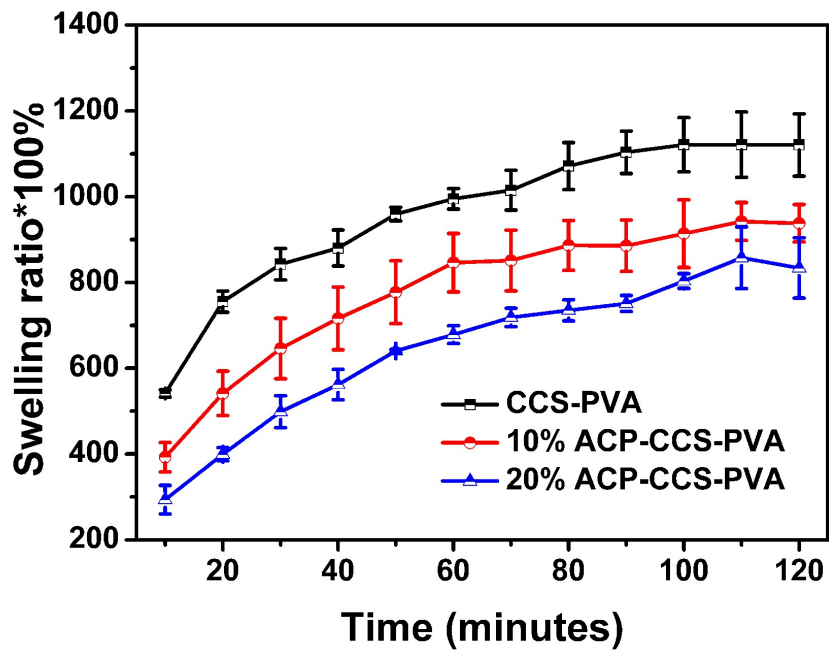


Fig. S5 The swelling ratio of composite membranes with different weight fractions of ACP.

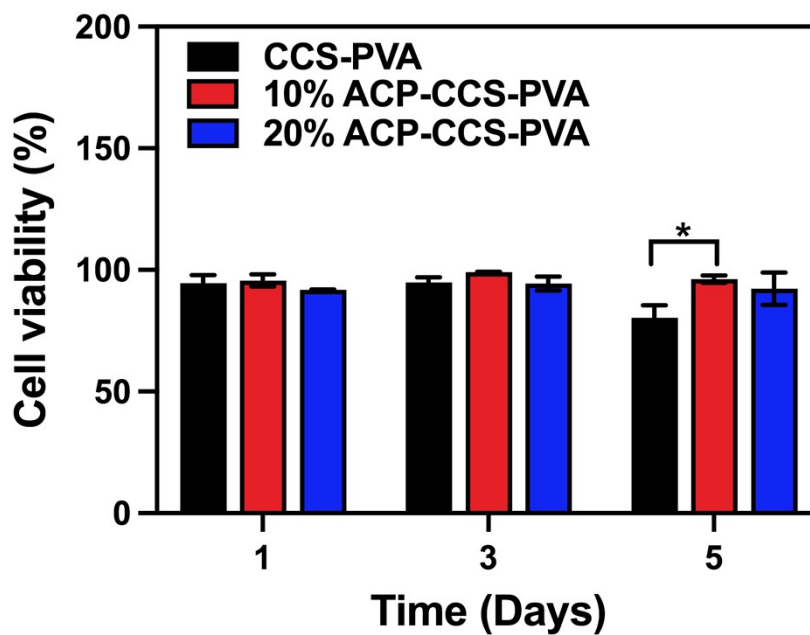


Fig. S6 Cell viability of CCS-PVA, 10wt%, and 20wt% ACP-CCS-PVA composite membranes (*P<0.05, n=3).

Table. S1 Genes and primer sequences

Gene	Primer	Sequence (5' - 3')
GAPDH	Forward	GGAGAGTGTTTCCTCGTCCC
	Reverse	ACTGTGCCGTTGAATTTGCC
ALP	Forward	ATCTTTGGTCTGGCTCCCATG
	Reverse	TTTCCCGTTCACCGTCCAC
RUNX2	Forward	AAATGCCTCCGCTGTTATGAA
	Reverse	GCTCCGGCCCACAAATCT
OPN	Forward	CTCACATGAAGAGCGGTGAG
	Reverse	TTCCTGGCTCTCTTTGGAA
OSX	Forward	CCCTTCTCAAGCACCAATGG
	Reverse	AGGGTGGGTAGTCATTTGCATAG