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Supporting Information

Repurposing Protein Aggregation into a Biofunctional BMP-2–Hyaluronic Acid Hydrogel for Sustained Drug Release and Accelerated Wound Healing

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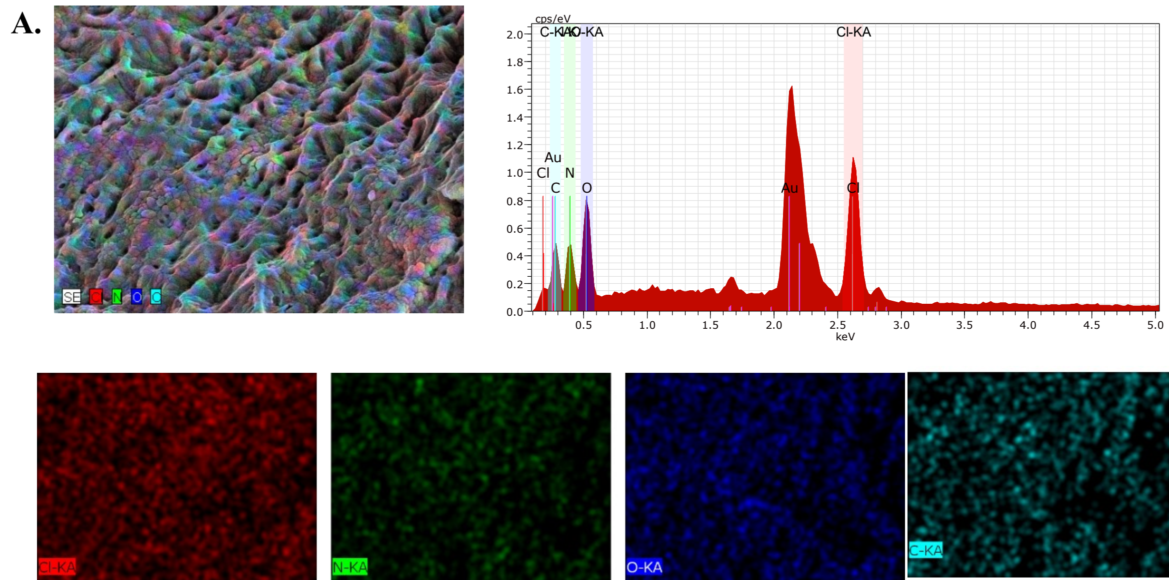
1. Elemental analysis of hydrogels from FESEM



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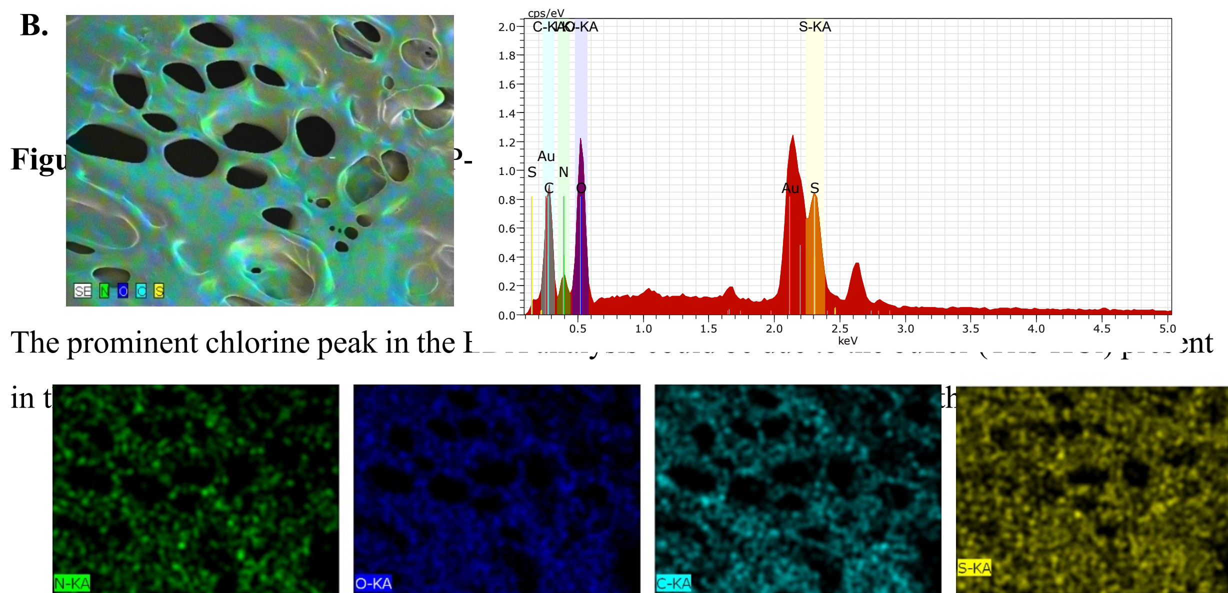

The prominent chlorine peak in the E ... nt

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2. Wound area calculation

The wound area calculations were done using sketchandcalc software. All images were taken in same DPI / same pixel resolution with same size in order to compare the wound area in different time scales.

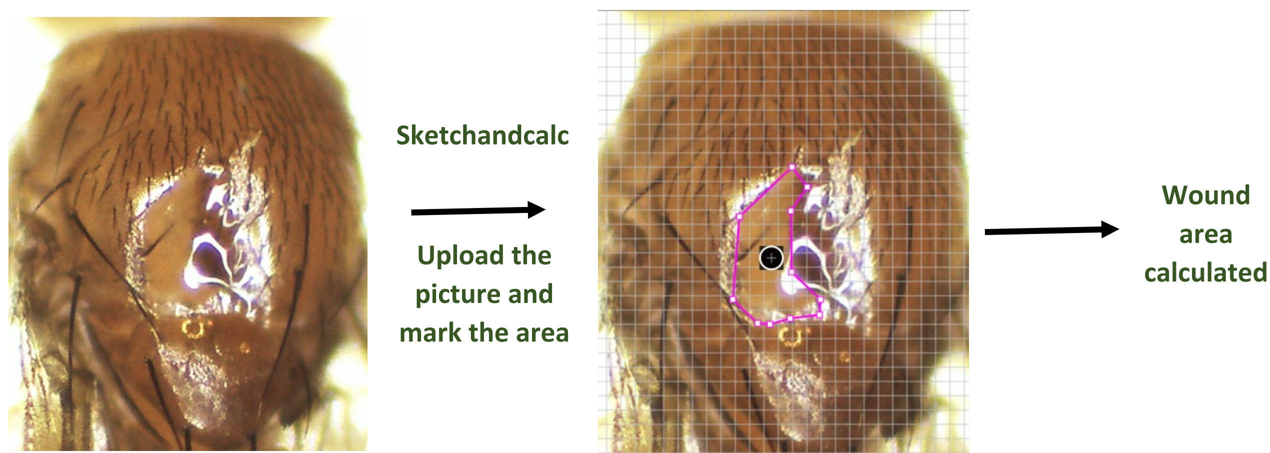


Figure S2. Procedure for wound area calculation.

3. Full spectra of absorbance data:

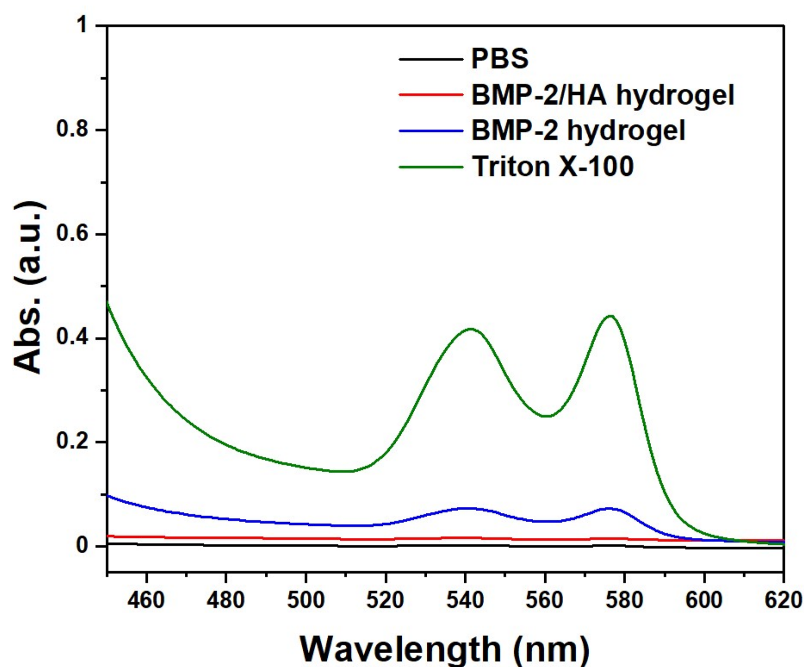


Figure S3. Absorption spectra of the hydrogel in the presence of RBCs.

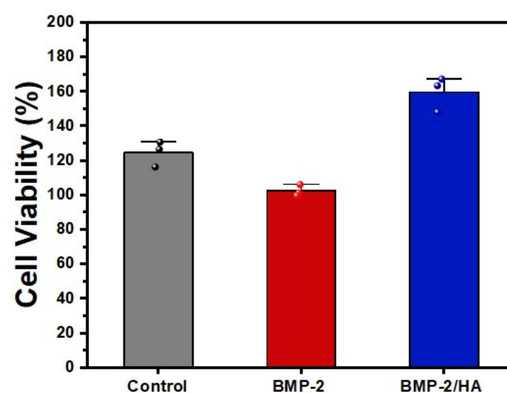
4. **Cell viability assay of L929 Cell line**

Method

The in vitro cytocompatibility of the synthesized nanobiocomposites was evaluated using the MTT colorimetric assay in the murine fibroblast L929 cell line. Cells were cultured under standard conditions (37 °C, 5% CO_2, humidified atmosphere) in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. For the assay, L929 cells were seeded in 96-well tissue culture plates at a density of 3×10^3 cells per well and allowed to adhere for 24 h. Subsequently, the culture medium was replaced with fresh medium containing nanobiocomposite suspensions at varying concentrations (5, 50, and 100 ppm), followed by incubation for an additional 24 h under identical conditions. After treatment, the medium was carefully aspirated, and 100 µL of MTT solution (0.5 mg mL^{-1} in serum-free medium) was added to each well. The plates were incubated for 4 h to allow metabolically active cells to reduce MTT into insoluble purple formazan crystals. Thereafter, the supernatant was removed, and 100 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formed formazan. The plates were gently shaken for 20–30 min to ensure complete dissolution. Absorbance was measured at 595 nm using a Bio-Rad iMark™ microplate reader. Cell viability was calculated by normalizing the absorbance of treated samples to that of untreated control cells, which were considered as 100% viable. All measurements were performed under identical experimental conditions to ensure reproducibility and accuracy.

Result

The extracts of the hydrogels (BHA and B) were prepared in triplicate and kept for a 24-hour



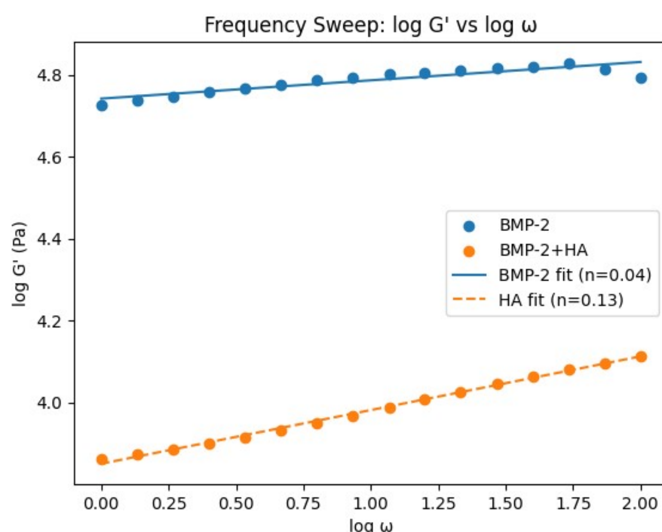
incubation period.

Figure S4. Cell viability test of hydrogel in L292 cell line.

It was observed that BHA and B are cytocompatibile as they have shown cell viability greater than 80%. BHA had shown greater cell proliferation than B in the 24-hour incubation period.

5. Fitting of the Rheology frequency sweep in Power law

Frequency sweep analysis shows that BMP-2 exhibits a weak frequency dependence of storage



modulus (n = 0.045), characteristic of a predominantly elastic gel.

Figure S5: Fitting of the Rheology in Power law

In contrast, BMP-2/HA displays a higher exponent (n = 0.13) with excellent fitting (R^2 = 0.996), indicating increased viscoelasticity and reduced network rigidity in the presence of hyaluronic acid. But in case both the storage modulus is greater than that of loss of modulation suggesting gelation.

Sample	n	R^2	Interpretation
BMP-2	0.045	0.818	Elastic gel (solid-like)
BMP-2 + HA	0.131	0.996	Viscoelastic (HA-modulated)