Electronic Supplementary Information

Enhanced skin penetration of berberine from proniosome gel attenuates pain and inflammation in a mouse model of osteoarthritis

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2. Methods

2.1 Reduction of the mechanical strength of S60-based proniosome gel using S80

The percentage mass ratio of S60 and S80 was optimized by a step-wise increment of the mass of S80 by 10 mg as shown in Fig S1. The different mixtures were used to formulate the proniosome gel and the flow behaviour of the proniosome gel was observed at different temperatures by heating the samples in the water bath for 1 minute. The percentage mass ratio of S60 and S80 was selected based on the flow behaviour of proniosome gel below 35 °C.

2.2 Quantification of berberine embedded in proniosome gel and encapsulated in niosomes

Quantification of berberine embedded in proniosome gel: 100 mg of the proniosome gel was hydrated with 20 mL PBS at 45 °C and the sample was vortexed for 5 minutes to ensure complete dissolution of the gel. An aliquot of the sample (100 µL) was taken and berberine was extracted using 100 µL of the solvent containing ACN: MeOH in 1:1 ratio. The mixture was vortexed, heated at 80 ˚C, and centrifuged to remove the surfactants. The absorbance of the supernatant was read with a microplate reader (Bio-Tek, Winoosky, VT, USA) at 346nm.

Quantification of berberine encapsulated in niosomes: 100 mg of the proniosome gel was hydrated with 20 mL PBS at 45 °C and the sample was vortex for 5 minutes to ensure a completed conversion of the gel into niosome vesicles. The sample was further dialysed in a dialysis bag, with a molecular weight cut-off (MWCO) at 3.5 kDa, at 4 °C to remove the unencapsulated berberine. An aliquot of the sample (100 µL) was taken out at various time points and the absorbance of the sample was read with a microplate reader (Bio-Tek, Winoosky, VT, USA) at 346nm to analyze the amount of berberine that was retained in the sample. The encapsulation efficiency of the niosome vesicles was determined upon confirming that the concentration of berberine in the sample remained constant in the dialysis bag.

2.3 Optical and fluorescence imaging of berberine in niosomes

An amount of 2 mg of berberine loaded proniosome gel was hydrated with 1 mL PBS and the sample was heated at 45 °C. The aliquot of the sample (10 µL) was taken out and the niosomes were observed under the Olympus-BX51 microscope (Olympus, Tokyo, Japan). Fluorescence images of berberine were taken using the green channel and the images were taken at 20x magnification. The size of the niosomes was measured using ImageJ software (National Institute of Health, Bethesda, MD, USA).

2.4 Release profile of berberine released from the proniosome gel

120 mg of various proniosome gel formulations loaded with berberine were placed in a holder. Each gel formulation was immersed individually in 15 mL of phosphate buffer saline solution at 32 °C. At each time point, an aliquot of solution (100 µL) was taken out and the sample was centrifuged to remove any remaining gel. The absorbance of the supernatant was read with a microplate reader at 346 nm to determine the concentration of berberine released from the formulation.

2.5 Western blot analysis

Protein samples from in vitro experiments were denatured, separated on 10% polyacrylamide gels (Bio-Rad Laboratories, Hercules, CA, USA), electro-blotted onto a nitrocellulose membrane (Bio-Rad), and probed with primary antibody followed by incubation with horseradish peroxidase (HRP)-coupled secondary antibody against the primary antibody. The primary antibodies for type II collagen (MAB8887; Merck Millipore, Billerica, MA, USA), MMP-13 (ab39012; Abcam, Cambridge, UK), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; #3638; Cell Signalling Technology, Danvers, MA, USA) were used. After incubating with the HRP-coupled secondary antibody, the protein bands were visualized using SuperSignal West Pico Chemiluminescent Substrate (Thermofisher Scientific) and then documented using the ChemiDoc™ MP System (Bio-Rad). Bands were quantified using Image Lab™ software (Bio-Rad) and plotted as graphs.

Supplementary Figures

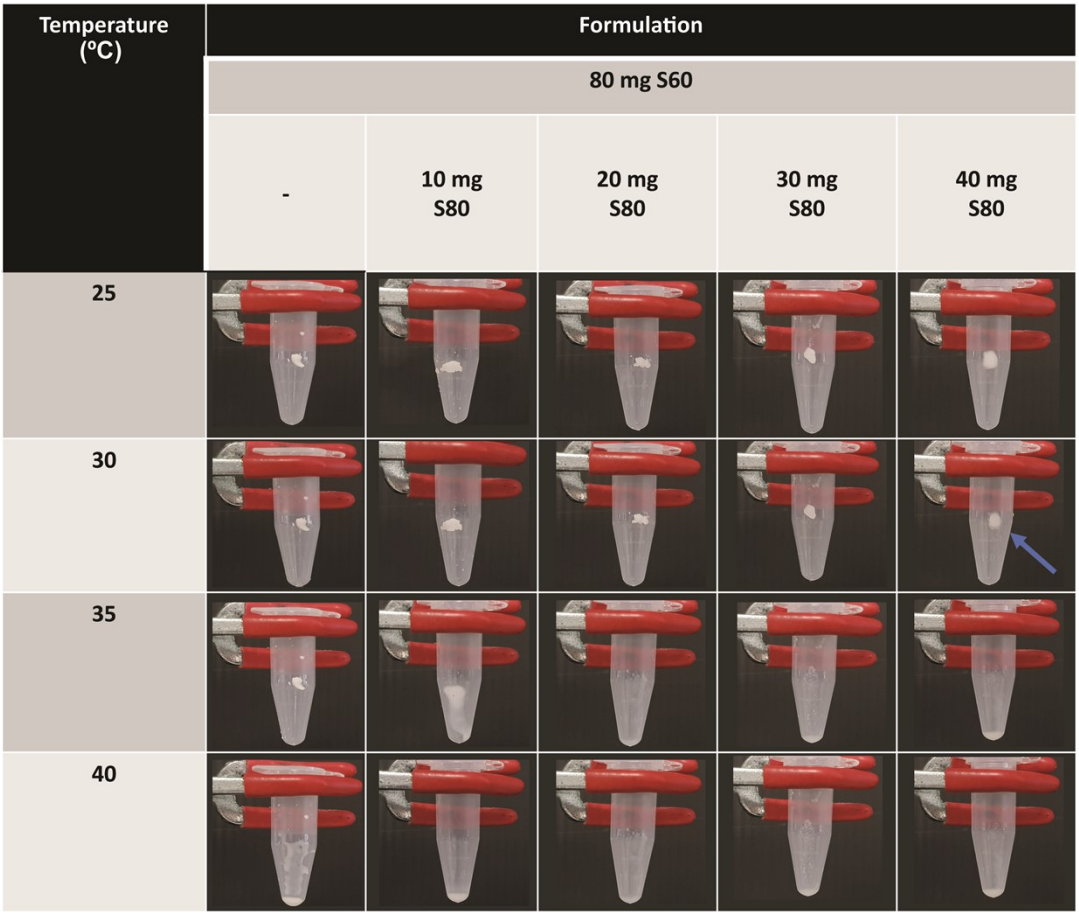

Fig S1 Optimizing the mass of S80 required to reduce the mechanical strength of S60-based proniosome gel. Proniosome gel were heated under the water bath for 1 minute and the flow of the gel formulation were observed after heating the samples at 1 minute.

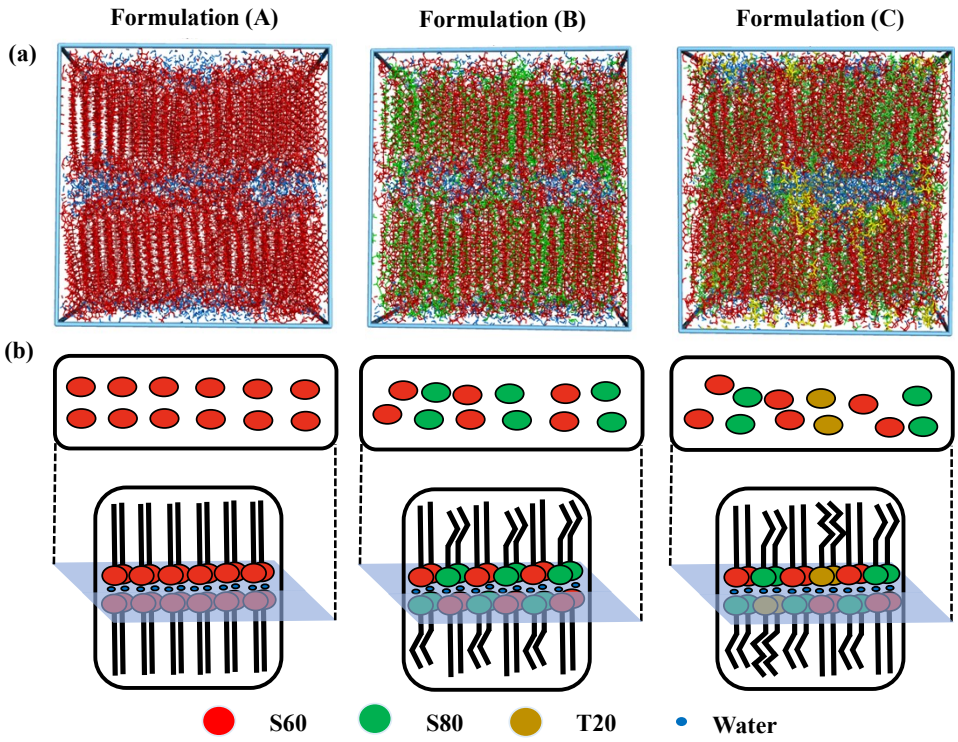

Fig S2 3D molecular simulation model of the bilayers in the proniosome gel. (a) Molecular simulation model of the bilayers in various proniosome gel formulation. (b) Schematic diagram illustrating the atomic density map that were derived from the simulation models. The schematic diagram illustrates the mechanism of S80 and T20 in disrupting the close packing of S60 in the bilayers.

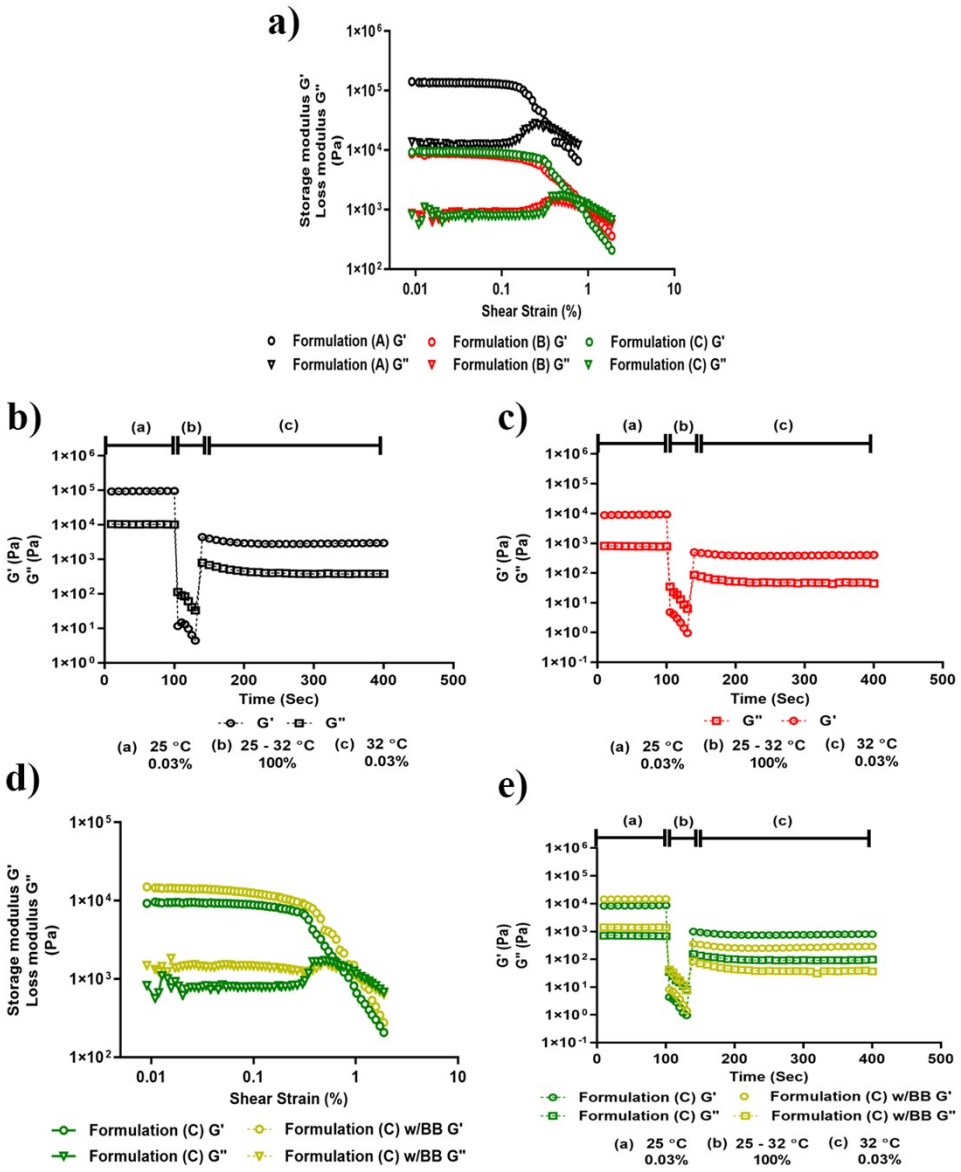

Fig S3 Rheological characterization of proniosome gel loaded with berberine. (a) Amplitude sweep measurement for all three proniosome gel formulations. (b-c) Dynamic step strain measurement of proniosome gels (A) and (B). (d) Amplitude sweep measurement for Formulation (C) loaded with berberine (Formulation (C) w/BB) in comparison to Formulation (C). (e) Dynamic step strain measurement of berberine loaded Formulation (C) in comparison to blank Formulation (C).

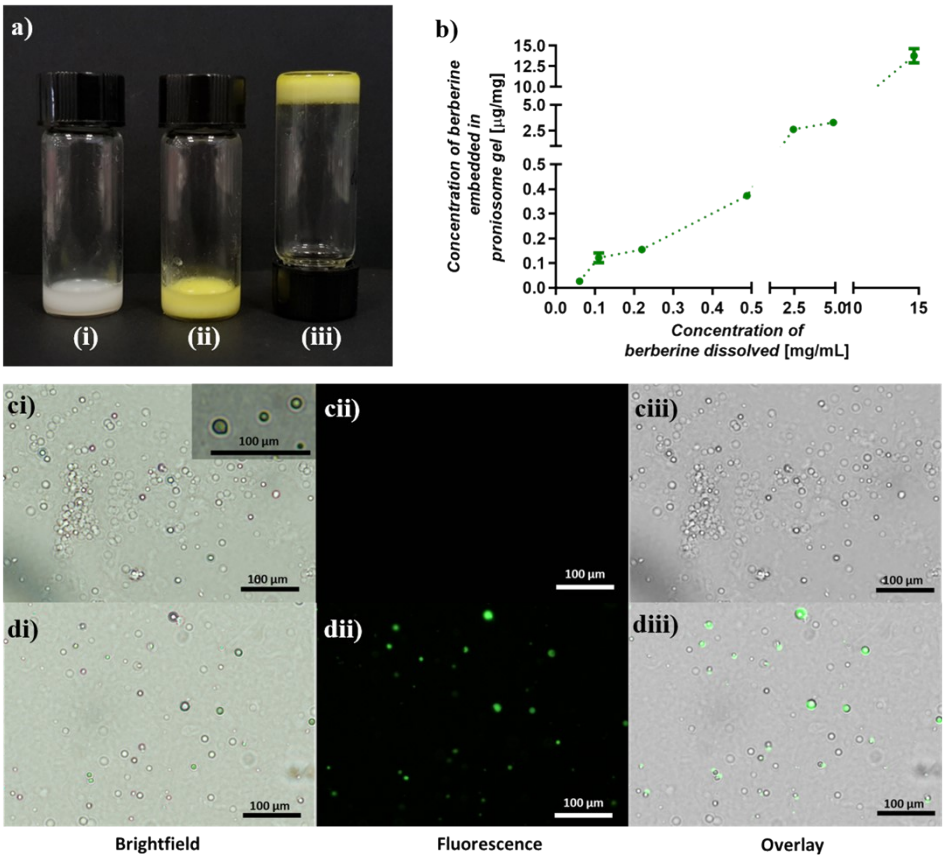

Fig S4 Characterization of berberine into proniosome gel (a) Digital images of the proniosome gel loaded with berberine with (i) Formulation (C) (ii) Formulation (C) loaded with berberine (iii) Formulation (C) loaded with berberine in an inverted position. (b) Increase loading of berberine in Formulation (C) of proniosome gel. (ci-iii) bright field, fluorescent and overlay images of niosomes. (di-iii) Bright field, fluorescent and overlay images of niosomes loaded with berberine. Scale bar of images is at 100 µm. Microscope images of niosome were taken at 20x magnification while the enlarged image of niosomes in (di) was taken at 50x.

Table S1 Quantification of berberine embedded in proniosome gel Formulations (A)-(C) and the percentage encapsulation efficiency of berberine in niosomes

Formulation	Amount of Berberine embedded in proniosome gel (mg)	Amount of berberine encapsulated in niosomes (mg)	Percentage encapsulation (%)
(A)	1746 ± 83.7	1167 ± 71.1	66.7 ± 1.3
(B)	1487 ± 76.9	968 ± 70.2	65.2 ± 4.5
(C)	1379 ± 85.9	833 ± 83.2	60.3 ± 2.4

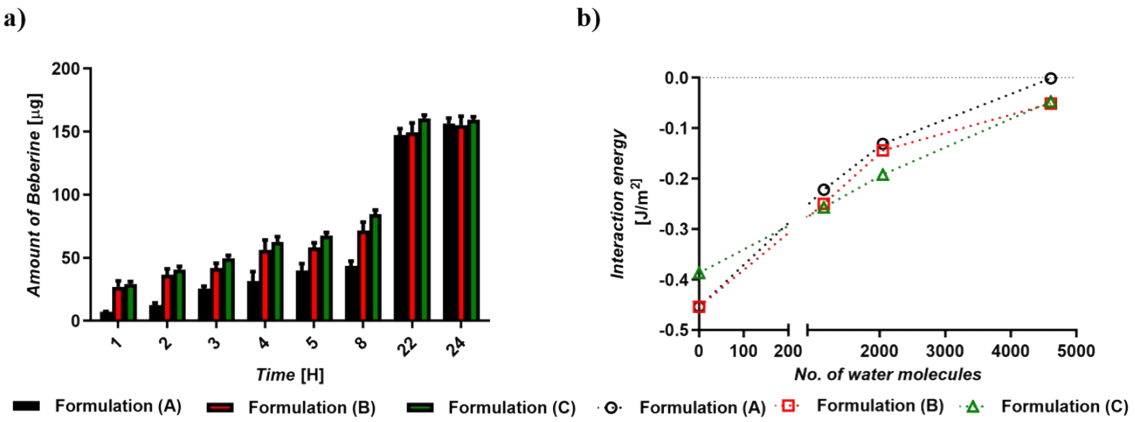

Fig S5 Correlating the release profile of berberine from various proniosome gel formulations with the molecular simulation data (a) Cumulative amount of berberine released from the proniosome gel formulations. (b) Interaction energy between two bilayers that represent the interaction between niosomes at varying amount of water molecules. Interaction energies were calculated based on molecular simulations.

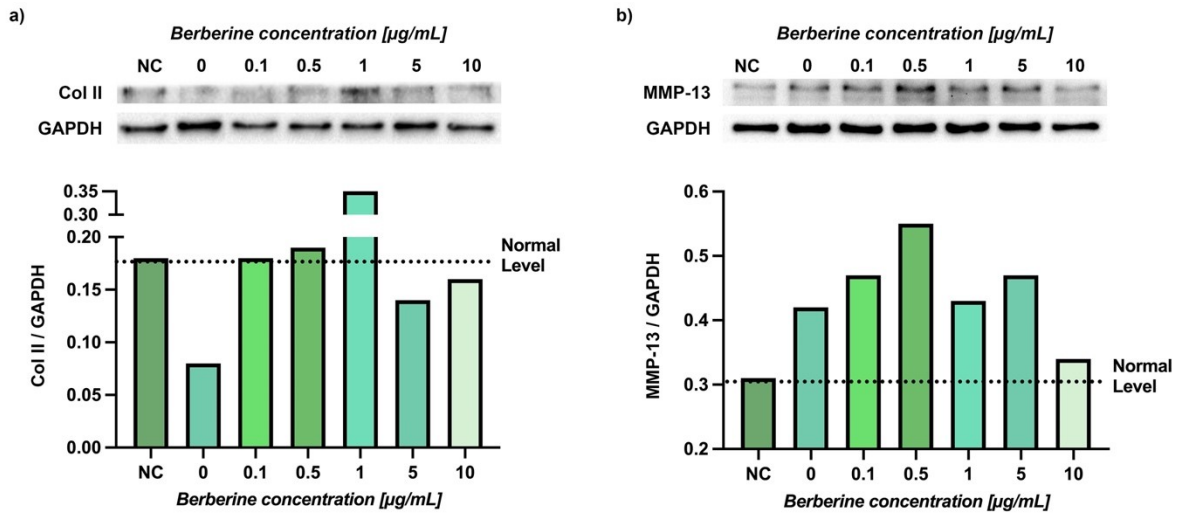

Fig S6 Effects of berberine on matrix biosynthetic activity of mouse chondrocytes in the presence of IL-1β and TNF-α. Western blot analysis showed that berberine restored the synthesis of (a) type II collagen (Col II) and suppressed the production of (b) MMP-13 in IL-1β/TNF-α stimulated chondrocytes. Image Lab™ software was utilized to quantify the protein bands and the data were normalized against GAPDH.

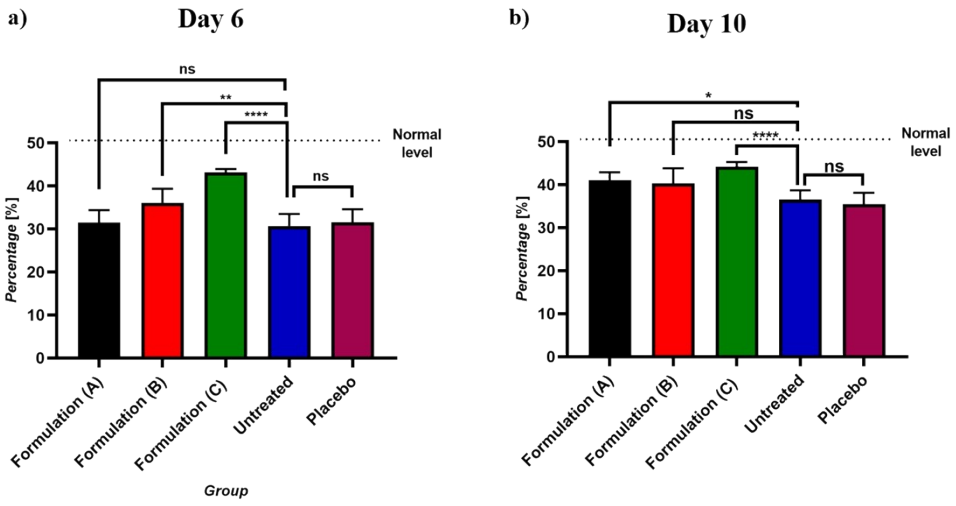

Fig S7 Percentage weight bearing ability of mice in various experimental groups at (a) day 6 and (b) day 10. *$P < 0.05$, **$P < 0.01$, ****$P < 0.0001$ and ns denotes statistically not significant.