Electronic Supporting Information

Fibroblast-Targeting Polymeric Nanovehicles to Enhance Topical Wound Healing though Promotion of PAR-2 Receptor-Mediated Endocytosis

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Supplementary experiments

Synthesis of a MEL-maleimide peptide linker

In the first step, a round-bottomed flask was charged with MEL (1 g), and purged with argon. Anhydrous methylene chloride (32 mL) and pyridine (367 µL) were sequentially added to the flask. A solution of 4-nitrophenylchloroformate (551 mg) in anhydrous methylene chloride (4 mL) was added to the mixture using a syringe, and the resulting solution was stirred for 2 min at room temperature. Saturated (aqueous) NH_4Cl was then added, and the solution was separated into aqueous and organic layers. The aqueous layer was extracted twice with methylene chloride (MC). The organic layers were combined, dried with $MgSO_4$, filtered, and concentrated using a rotary evaporator. The resulting residue was purified by flash column chromatography on silica gel (EtOAc:n-hexane = 1:1) to obtain the orthoformate intermediate (750 mg) as a colorless oil. In the second step, a round-bottomed flask was charged with an orthoformate intermediate (100 mg). The flask was evaporated and refilled with argon. Acetonitrile (2.8 mL) was added, followed by addition of N,N-diisopropylethylamine (73 µL). N-(2-Aminoethyl) maleimide trifluoroacetate salt (53 mg) was added to a single portion, and the reaction was stirred for 18 h at room temperature. The solvent was then removed under reduced pressure. The residue was dissolved in MC, and concentrated using a rotary evaporator. The resulting residue was purified by flash column chromatography on silica gel (EtOAc:n-hexane = 2:1) to obtain thr MEL-maleimide linker (56 mg) as a yellowish oil.

In vitro wound scratch assay

Wound healing performance was evaluated by using an *in vitro* scratch assay. HDF cells were seeded at a density of 4×10^3 cells per well in Ibidi culture inserts 2 well (Ibidi GmbH, Germany), with a 500 µm cell-free gap placed in a 12-well plate, and incubated for 24 h. Thereafter, the Ibidi Culture-Insert was removed, and the cells were gently washed three times with PBS to remove debris. The cells were then treated for 24 h with a 1% FBS medium as a control, a curcumin solution in DMSO, and $CPNV_{KTTKS}$ diluted 1% FBS medium ([curcumin]=5 µM). Wound closure by cell migration was photographed with an optical microscope (Axio Vert.A1 Bio, ZEISS, Germany) during incubation for 48 h. *In vitro* wound healing was evaluated by the percent reduction of the initial scratch area according to the incubation time.

Supplementary data

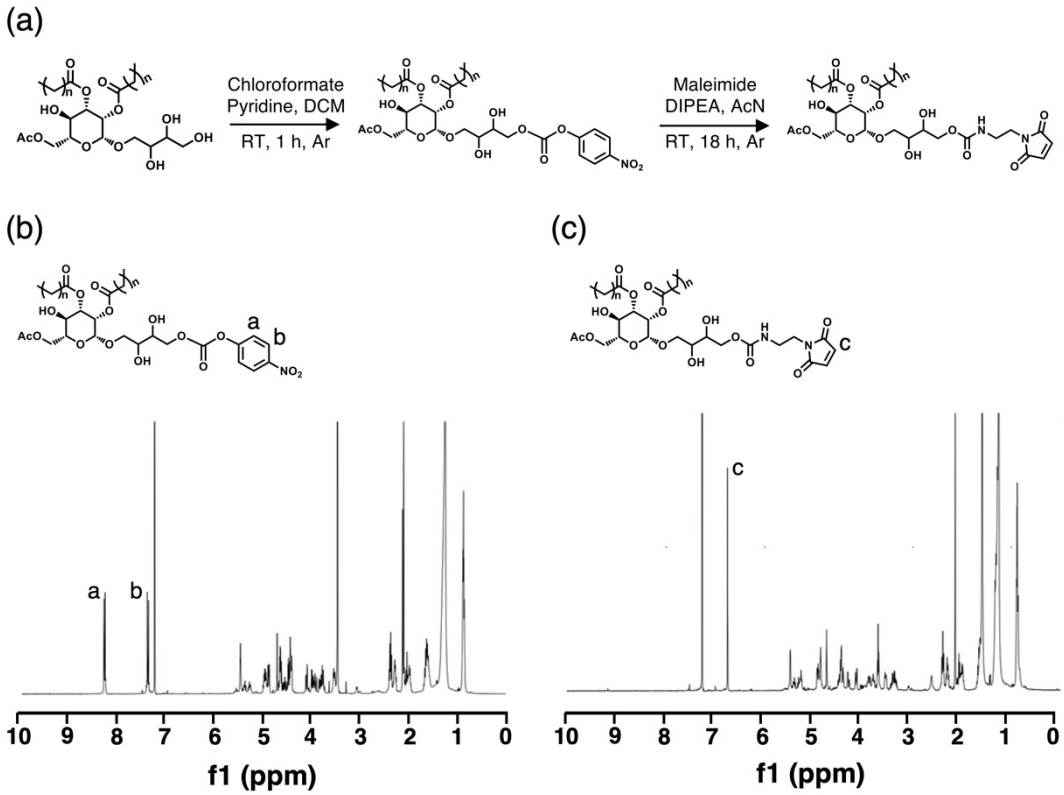

Figure S1. (a) Synthesis of a MEL-maleimide linker. ^{1}H NMR spectra (b) before and (c) after incorporation of maleimide to MEL.

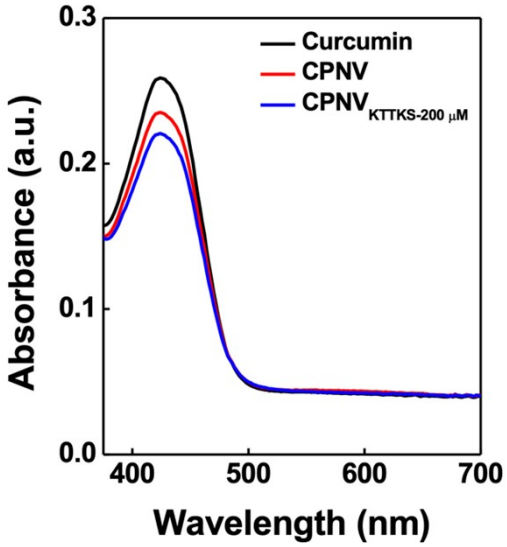

Figure S2. UV-vis spectra of $CPNV_{KTTKS}$ loaded with the same amount of curcumin according to the fabrication recipe.

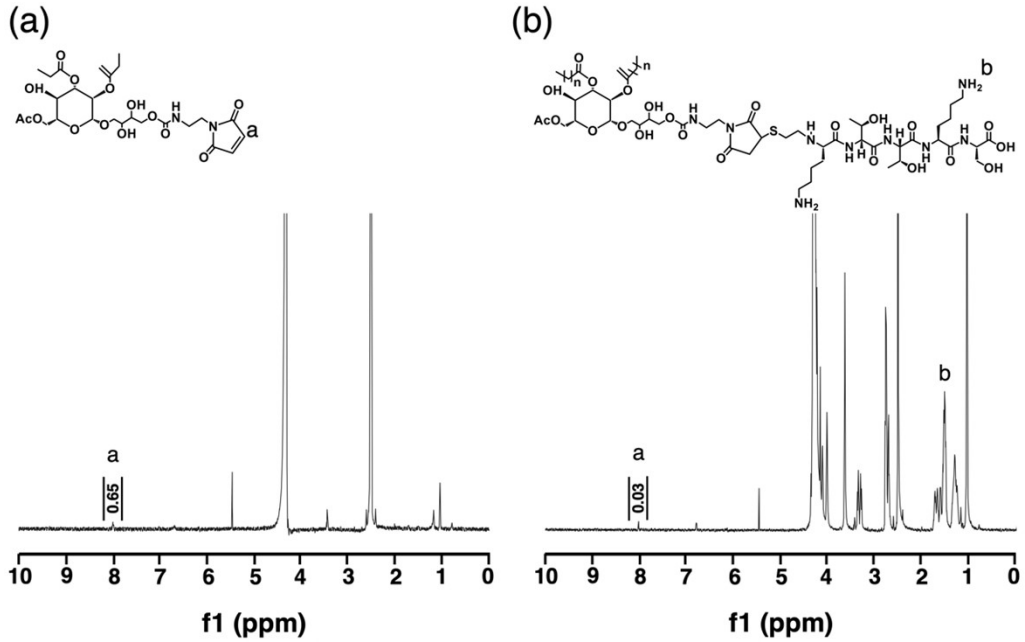

Figure S3. Chemical structure and analysis of ^{1}H NMR spectra of (a) MEL-maleimide linker and (b) KTTKS conjugated MEL-maleimide linker. The NMR solvent system was conditioned with the mixture of DMSO-d_6 and D_2O with a ratio of 5:3 (v/v).

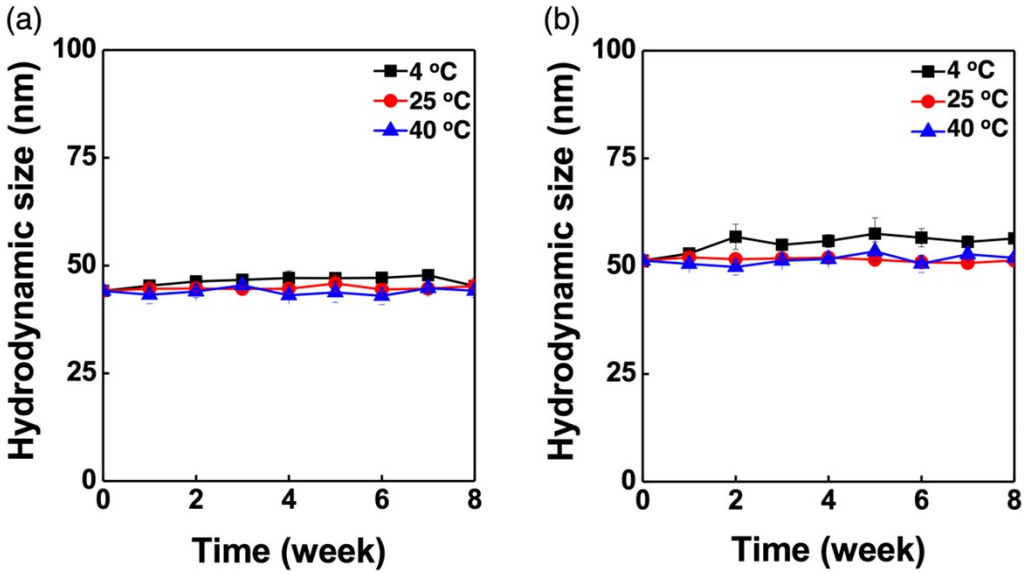

Figure S4. Long-term storage stability of PNV_{KTTKS} dispersions for 8 weeks at different storage temperatures: (a) $PNV_{KTTKS\text{-}200\ \mu M}$ and (b) $CPNV_{KTTKS\text{-}200\ \mu M}$. The PNV_{KTTKS} was prepared with PEO_{5k}-*b*-PCL_{2k}.

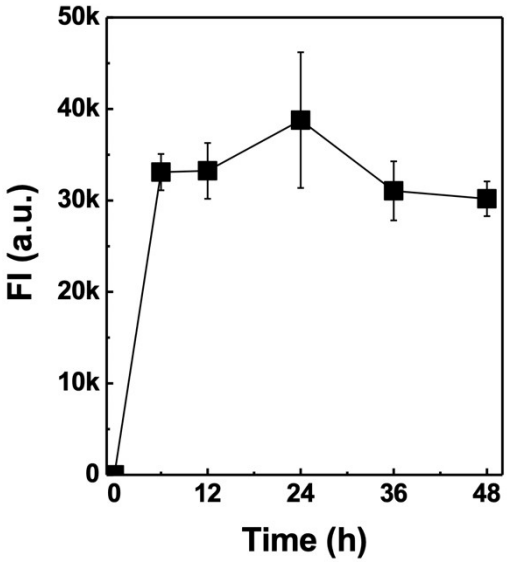

Figure S5. Quantitative cellular uptake of Nile red-loaded PNV_{KTTKS} prepared depending on incubation time. PEO_{5k}-*b*-PCL_{2k} was used in this case. Error bars represent standard deviation of the mean (n = 4)

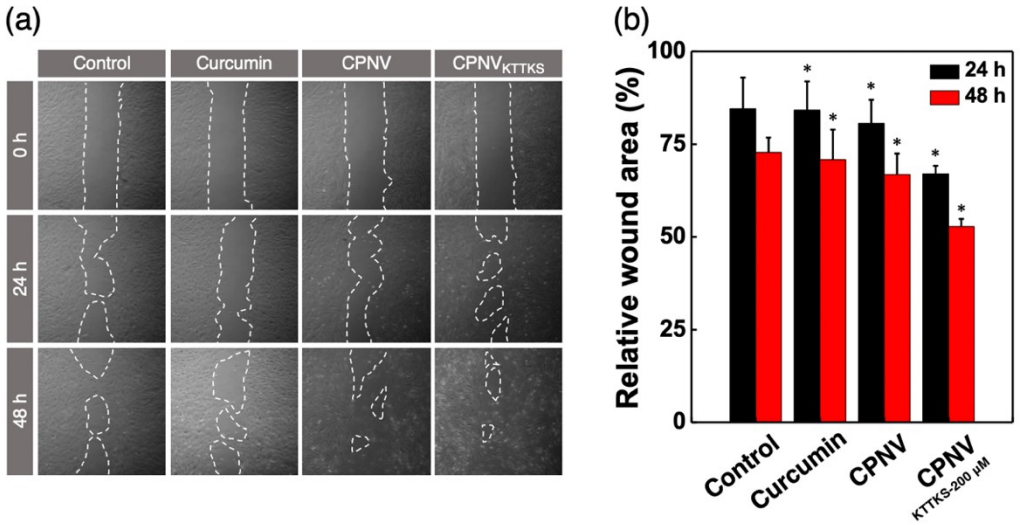

Figure S6. *In vitro* scratch assay of HDF cells. (a) Representative micrographs of HDF cells after treatment with $CPNV_{KTTKS\text{-}200\ \mu M}$ at different incubation periods at 37 °C. (b) Quantification of relative wound areas based on the micrographs. Error bars represent standard deviation of the mean (n = 3) (*$p < 0.05$ compared to untreated cells, one-way ANOVA).