Melittin-encapsulating Peptide Hydrogels for Enhanced Delivery of Impermeable Anticancer Peptides

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Experimental Section

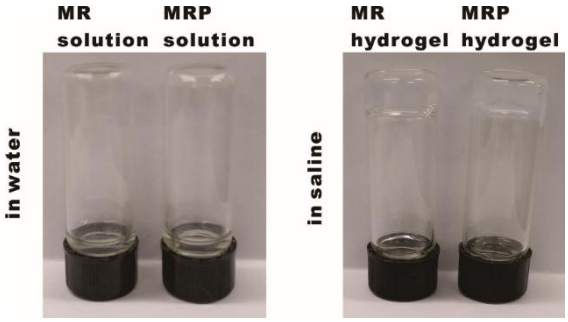
Materials and cell lines. KLA $(KLAKLAKKLAKLAK)_D$, Melittin (GIGAVLKVLTTGLPALISWIKRKRQQ), and $RADA_{28}$-melittin fusion peptide (Ac-RADARADARADARADARADA-GG-GIGAVLKVLTTGLPALISWIKRKRQQ-NH2) were synthesized by Bankpeptide Ltd. (Hefei, China).

CT-26 cells were purchased from the Chinese Type Culture Collection (CTCC) and were maintained in RPMI 1640 medium (Gibico) with 10% FBS (Gibico) and 100 ug/mL antibiotics (penicillin and streptomycin) in an atmosphere of 5% CO_2 at 37 ℃.

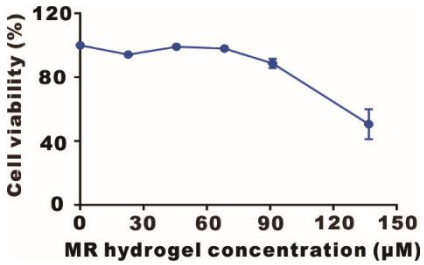
Preparation of MRP hydrogel. The KLA solution (10 mM) was obtained by dissolving 5 mg of KLA powder in ultrapure water at room temperature. To prepare MR hydrogel, 10 mg MR peptides were dissolved in 1 mL 0.9% NaCl solution and kept at 4 °C overnight. In the same way, 10 mg MR peptides and different dosages of KLA peptide were dissolved in 1 mL 0.9% NaCl solution and kept at 4 °C overnight to prepare MRP hydrogel. All procedures were performed under sterile conditions.

Cell viability assay. CT26 cells (5000 cells/well) were seeded in 96-well plates with 100 μL of culture medium and cultured at 37 °C and 5% CO_2 overnight. Then various concentrations of MR (concentration: 0-15 mg/mL) were added in quadruplicates to the culture media. After incubation for 24 at 37 °C, the cell viability was determined using the CCK8 assay (Beyotime).

Statistical Analysis. Data in this study are presented as mean ± standard error of the mean (SEM). For *in vitro* studies, unpaired Student t-tests were used for statistical comparison. Tumor growth was analyzed using the Kaplan-Meier method. Statistical significance was expressed as * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$.



Supporting Figure 1. Procedure of gelation under physiological conditions.



Supporting Figure 2. Cell viability measurement using the CCK-8 assay after treatment with MR hydrogel for 24 h. Data are presented as the mean ± SEM (n = 4).