A novel delivery vehicle for copper peptides

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Materials.

Betaine ,DL-Mal and Tar acids were from Sigma-Aldrich (St. Louis, MO, USA); Dulbecco's modified Eagle's medium (DMEM) from Gibco (Grand Island, USA); Fluorescein isothiocyanate labed GHK-cu from Shanghai Apeptide Co.,Ltd.(Shanghai, China); SA-β-Gal and Annexin V-FITC apoptosis detection kit from Beyotime Biotechnology(Shanghai, China).

Characterizations.

Spectroscopy and Fourier-transform infrared (FTIR; Thermo Scientific Nicolet iS 50, USA) spectroscopy operated in attenuated total reflection mode. The chemical structures of ILs dispersed in D_2O were characterized using proton nuclear magnetic resonance (1H NMR; Bruker Avance III 400, USA). The size distribution and zeta potential of ILs were evaluated using the Zetasizer Nano ZS (Malvern, UK). Thermogravimetric analysis (TGA) was performed in an N2 atmosphere at temperature increments of 10 °C min-1 using a thermal analyzer (Netzsch STA 449F3, Germany). Differential scanning calorimetry (DSC) (Mettler Toledo DSC-3, Switzerland) measurements were conducted using N2 and the system was cooled using liquid nitrogen on a thermal analyzer at temperature increments of 10 °C min-1. The absorbance of samples was recorded using ultraviolet-visible (UV-Vis) spectroscopy (PerkinElmer, Lambda 365, USA). The morphology of ILs was observed by transmission electron microscopy (TEM, 120 kV , FEI Tecnai G2 T12, Netherlands) and scanning electron microscopy (SEM, 5 kV , Hitachi SU8010, Japan). High-performance liquid chromatography (HPLC) measurements were conducted using the Agilent 1100 equipped with a quaternary pump, a diode array detector (DAD), an autosampler, and a thermostatted column compartment (Agilent, Santa Clara, USA).

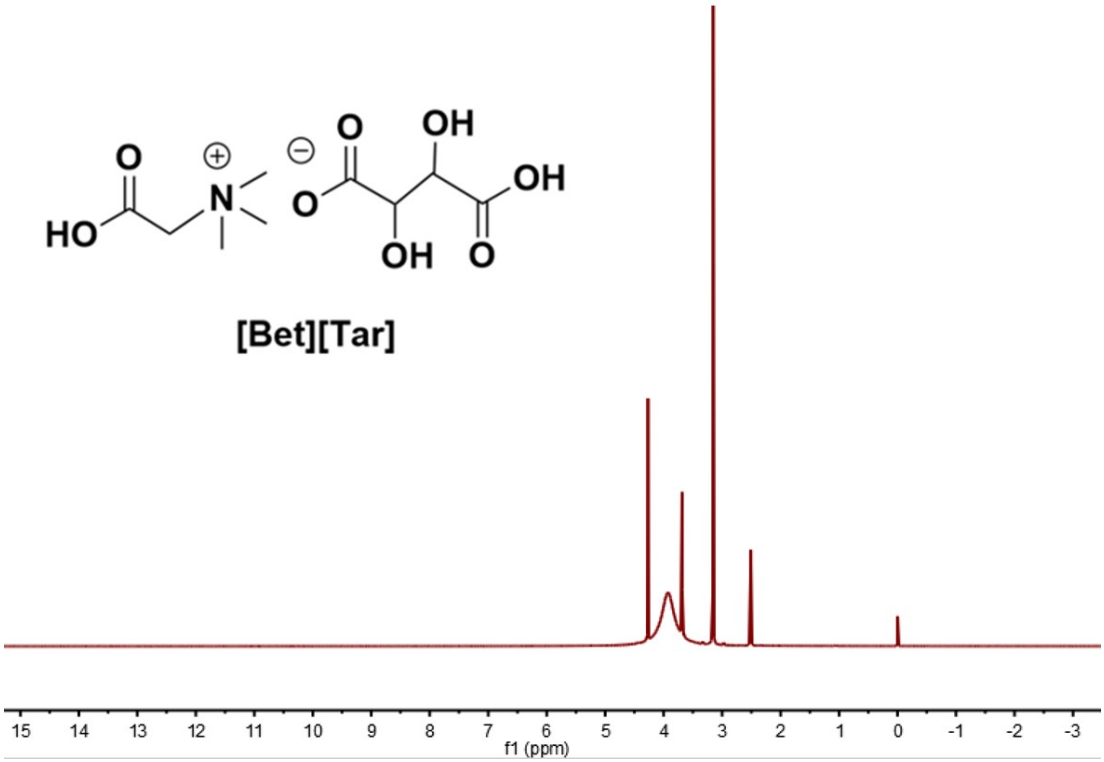

Figure S1. ^{1}H NMR spectrum of [Bet][Tar].

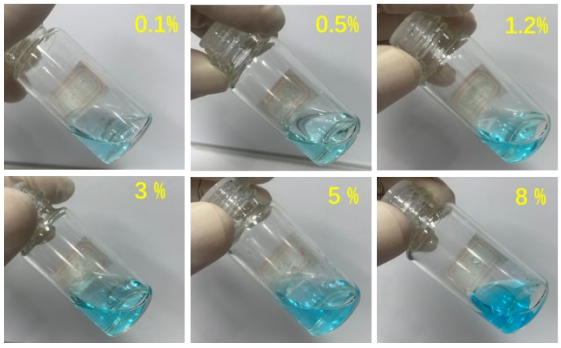

Figure S2 Physical picture of different GHK-Cu contents after 160 days of storage.

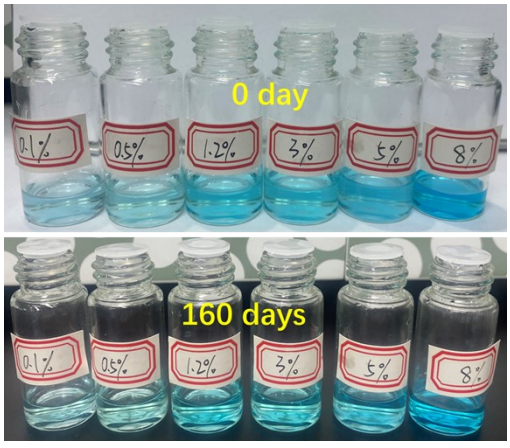

Figure S3 Comparison graph of 0 and 160 days.

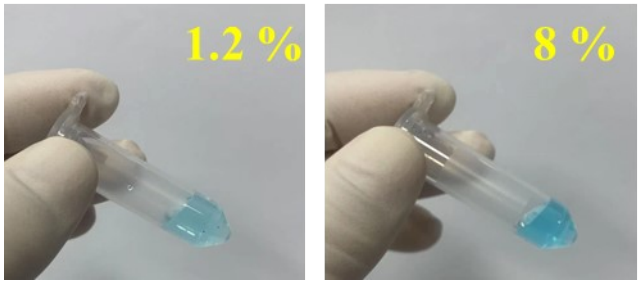

Figure S4 Physical picture after centrifugation at 4000rpm for 15 minutes.

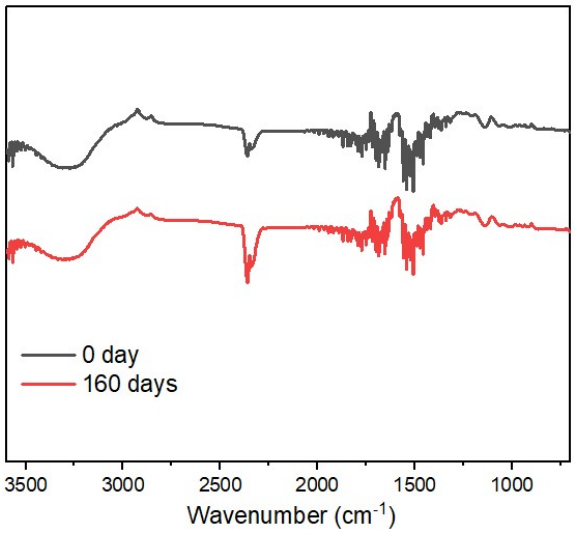

Figure S5 FTIR comparison graph after 160 days of storage.

Form of material	Delivery methods	Delivery efficiency	Disadvantages
ILs	Transdermal	High	Not yet
Microneedle	Transdermal	High	With trauma
Graft trim	Injections /Transdermal	Low	Process complexity
Gel Coat	Transdermal	Low	Process complexity /Low drug loading
Cosmetic formulations (lyophilized powders, masks, serums, etc.)	Transdermal	Low	Complex composition /Easily deactivated

Table S1 Comparison of different transdermal delivery methods of GHK-Cu.

GHK-Cu content (%)	Size		PDI	
	0 day	160 days	0	160 days
1.2%	63.7	65.6	0.125	0.133

5%	67.2	68.3	0.136	0.141
8%	69.1	70.3	0.129	0.137

TableS2 Comparison of particle size and PDI variation.