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Introduction:

Copper peptide is a kind of active peptide with the effect of skin repair^[1]. Copper peptide has strong function, but due to its high hydrophilicity, it is difficult to transport directly from vitro to vivo. Some studies have shown that copper peptide has poor biological stability^[2]. In order to improve the bioavailability of copper peptide, structural modification of copper peptide to enhance its stability and permeability has become the main research direction^[3-4]. We introduced the innovative lipophilic group palmitic acid to modify and transform the Lys side chain of GHK-Cu, and successfully synthesized a new copper peptide Pal-GHK-Cu. In this article. We proved that the novel copper peptide can effectively improve the retention in the skin layer, and effectively improve the bioavailability of copper peptide. And through in vitro cell toxicity and efficacy tests, verified the safety of the new copper peptide and skin repair efficacy, enrich the research of copper peptide.

Materials & Methods:

Synthesis of Pal-GHK-Cu (patent pending)

The amino acid sequence of Pal-GHK-Cu assigned to be a novel copper peptide (the amino acid sequence: H-Gly-His-Lys(Pal)-OH.Cu), was synthesized by using solid-phase peptide synthesis (SPPS) method.

Characterization of Pal-GHK-Cu

The HPLC (Agilent,1100) and MS(Agilent,6120) instruments were used to characterize the prepared Pal-GHK-Cu.

Transdermal Penetration Test

The permeability test was carried out by Franz diffusion cell. LC-MS/MS (Agilent 1290 Infinity II /Agilent 6460 Triple Quad LC/MS) was used to qualitatively and quantitatively analyze the novel copper peptides in the receiving fluid and skin samples collected at a predetermined time. The permeability of copper peptide was compared with that of unmodified copper peptide [19-20].

Clinical studies

We selected 15 female volunteers with the clinical study who met the requirements for wrinkles of 35-55 years old. The essence containing 2.5mg/l of the novel copper peptide was applied to the the corner of the eye once every morning and evening for 4 consecutive weeks, and the data of D0, D14 and D28 days were recorded. The improvement of periocular wrinkles was evaluated using Primos (LMI Technologies GmbH).

References:

- [1] Pickart L and M. M. Thaler, Tripeptide in human serum which prolongs survival of normal liver cells and stimulates growth in neoplastic liver, Nature New Biol, 1973, 243, 85-87.
 [2] Endo T, Miyag M, et al. Simultaneous determination of glycyl-L-histidyl-L-lysine and its metabolite L-histidyl-L-lysine in rat plasma by high-performance liquid chromatography with post column denaturation. J Chromatogr (B), 1977, 692: 37-42.
 [3] PENG K J, DAI K X, et al. Preparation and Characterization of GHK-Cu Liposome and its Effects on Hair Growth in Mouse Alopecia Model. China Academic J. 2013, 32 (8): 985-989.
 [4] Pickart L. Cosmetic and Skin Treatment Composition. United States Patent 5135913, Aug.4 1992.

Results & Discussion:

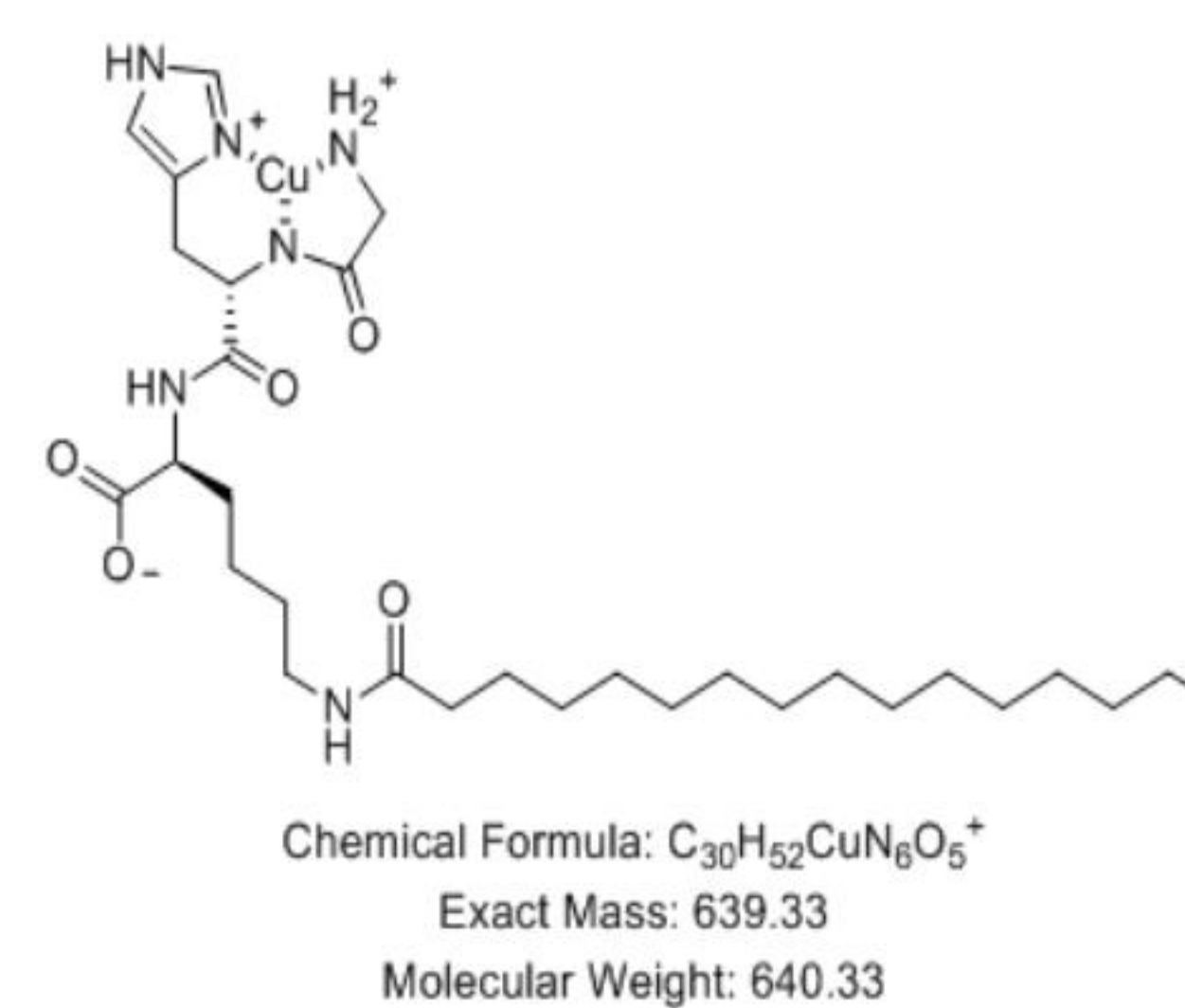


Fig.1. The chemical structure of Pal-GHK-Cu

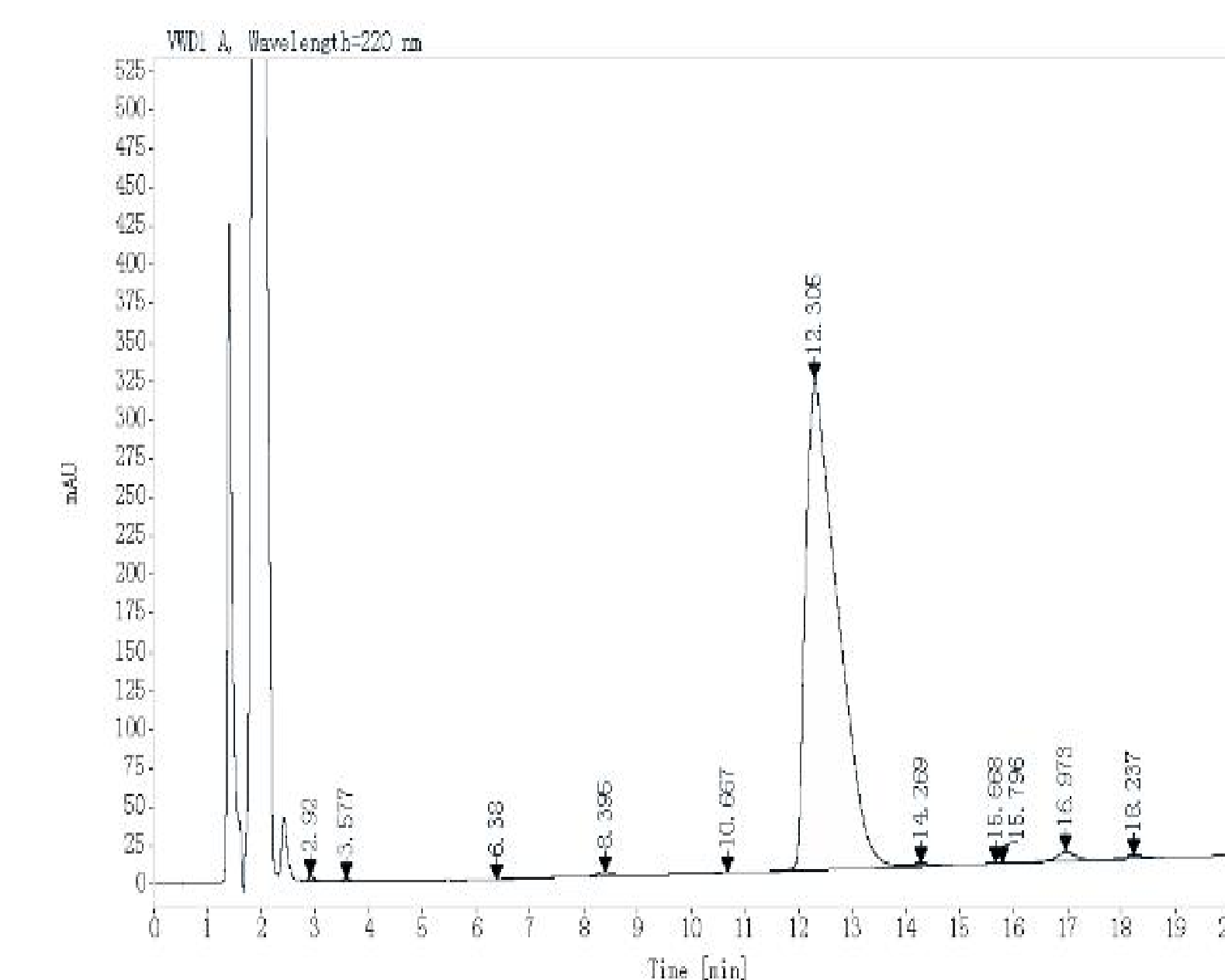


Fig.2. The HPLC elution data of Pal-GHK-Cu

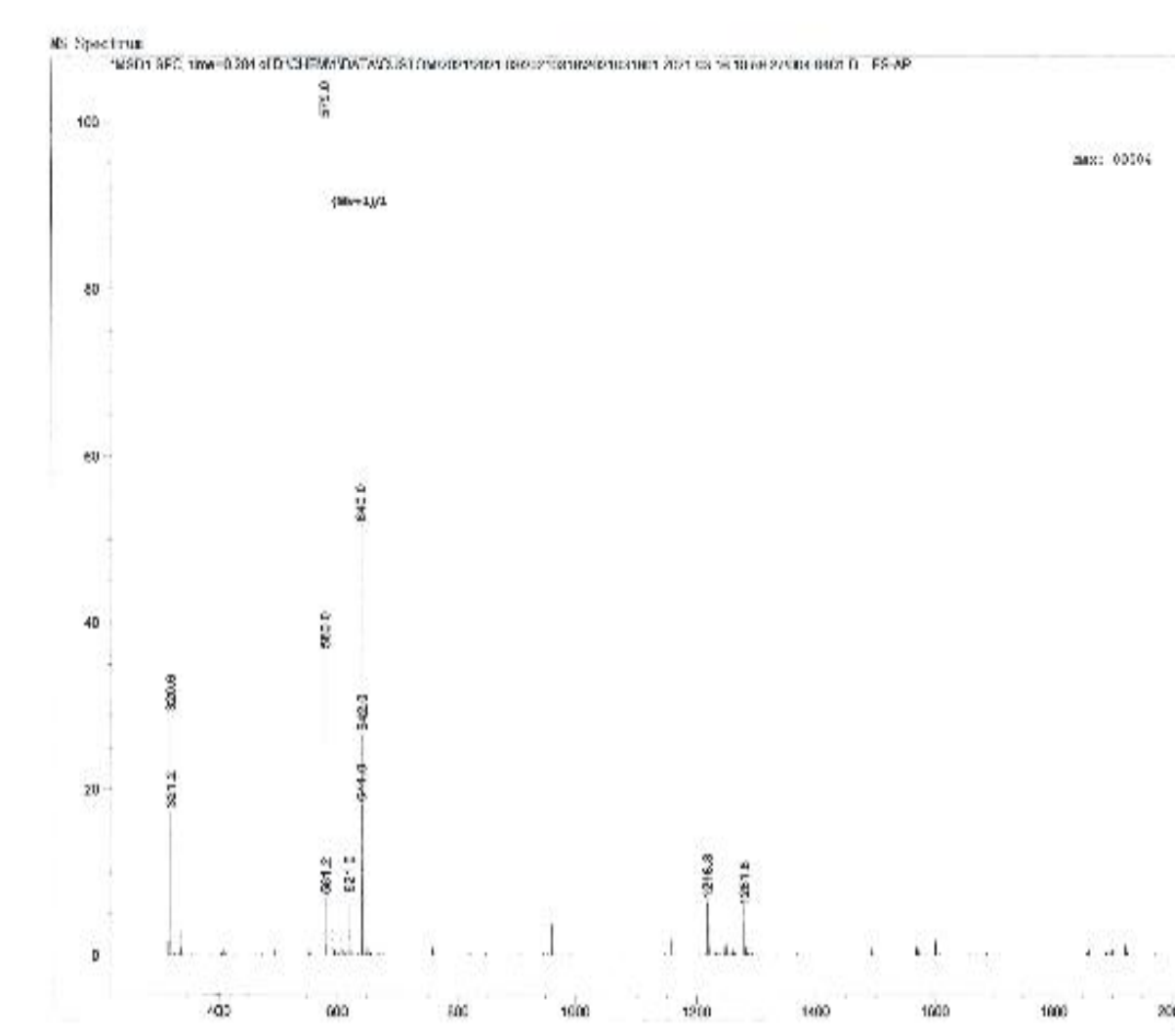


Fig. 3. The MS elution data of Pal-GHK-Cu

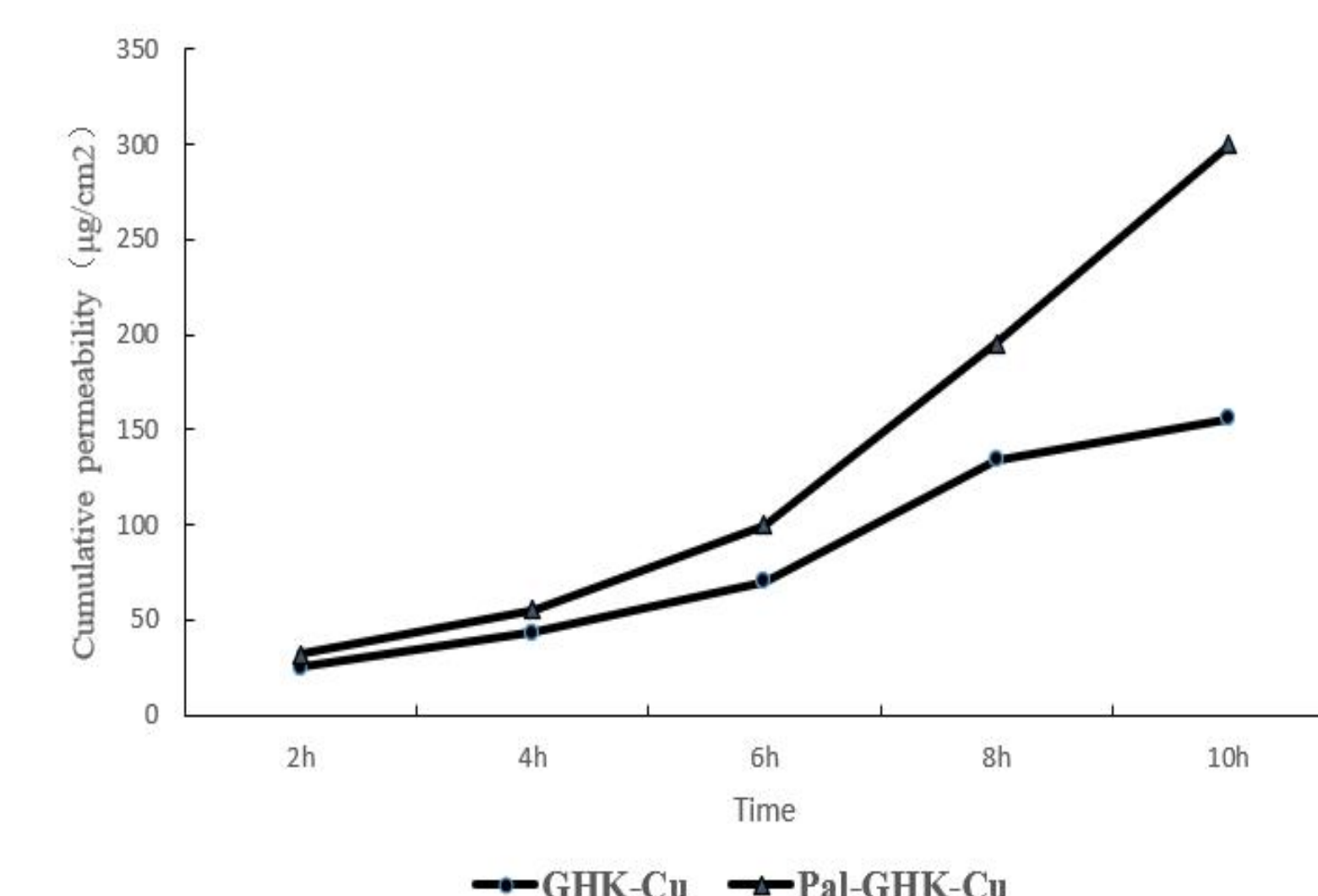


Figure.5. Calculation results of cumulative skin permeability

The Corner of Eye	D0	D14	D28	D0-D14	D0-D28	14-day improvement rate	28-day improvement rate
Mean depth (um)	66.67	59.44	58.00	7.22	8.67	10.83%	13.00%
Quantity	140.67	98.56	93.67	42.11	47.00	29.94%	33.41%
Area (mm ²)	94.29	84.48	81.88	9.81	12.42	10.41%	13.17%
Length (um)	141.89	132.67	130.67	9.22	11.22	6.50%	7.91%
Volume (mm ³)	6.25	5.08	4.86	1.17	1.40	18.71%	22.33%

Table.5. Results of clinical studies

Conclusions:

We successfully synthesized the novel copper peptide Pal-GHK-Cu by solid phase synthesis method. The results of penetration test showed that the novel copper peptide has better skin permeability. An extract containing 2.5mg/ L of the novel copper peptide was effective in reducing the depth of wrinkles and significantly removing crow's feet. Therefore, the novel copper peptide has potential applications in medicine, especially in skin cosmetology, including in the field of reparation.