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"BluX Supertide: The Ultimate Fusion Carrier of Hyaluronic Acid-Arginine Derivatives and GHK-Cu Peptide for Ageless, Revitalized Skin"

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1. Introduction

Glycyl-L-histidyl-L-lysine-copper (GHK-Cu) is a naturally occurring tripeptide–copper complex widely recognized for its potent anti-aging properties and skin-regenerative activities [1]. Functioning as both a signaling molecule and a carrier peptide, GHK-Cu promotes collagen and elastin synthesis in dermal fibroblasts, accelerates wound healing by enhancing angiogenesis and epithelial proliferation, and upregulates superoxide dismutase to mitigate oxidative stress [2-7]. However, its application in topical cosmetics is limited by three fundamental barriers: (1) the inherent instability of the copper ion (Cu)–peptide coordination; (2) poor penetration across the stratum corneum into the viable epidermis and dermis; and (3) the absence of a sustained release mechanism to maintain therapeutic concentrations over time.

Various transdermal delivery strategies—ranging from chemical modifications and covalent or physical crosslinking of polymers to assembly-based carriers such as liposomes, microcapsules, and nanogels—have been explored to enhance the bioavailability of cosmetic actives [8,9]. Nevertheless, these approaches are often constrained by three interconnected challenges: (1) loading efficiency typically decreases as the polarity or coordination complexity of the active ingredient increases; (2) high-molecular-weight carriers and hydrophilic cargos exhibit limited skin penetration; and (3) achieving controlled, sustained release without inducing residual toxicity or compromising stability remains difficult. For example, while anionic phospholipid vesicles can destabilize metal–peptide complexes such as GHK-Cu, high-pressure homogenization methods often produce heterogeneous particles with inconsistent release profiles.

Hyaluronic acid (HA) and its derivatives have emerged as leading biocompatible carriers for topical actives, owing to their abundant hydrophilic groups, high water retention capacity, and ability to form three-dimensional networks ideal for encapsulating and protecting sensitive molecules [10-14]. Early HA-based delivery systems primarily relied on covalent crosslinking (e.g., hydrazone, disulfide, PEG linkages) to generate nanogels with enhanced mechanical strength and loading capacity. However, these approaches often involve multi-step syntheses and carry risks of residual crosslinker toxicity. More recently, physical and supramolecular strategies—utilizing noncovalent interactions such as hydrogen bonding and electrostatic assembly—have enabled the construction of dynamic HA networks capable of entrapping hydrophilic cargos without the use of toxic reagents. Although these systems offer improved safety profiles and tunable release properties, achieving the simultaneous optimization of network stability and efficient transdermal penetration remains a major challenge.

To overcome these limitations, we report herein a novel physically dynamic supramolecular network carrier composed of HA and dihydroxypropyl arginine hydrochloride (DPAH). This HA–DPAH system, termed BluX Supertide, spontaneously assembles via noncovalent interactions into a four-dimensional scaffold capable of (1) preserving the integrity of GHK-Cu coordination, (2) enhancing the transdermal penetration of both high-molecular-weight HA and GHK-Cu, (3) providing sustained release kinetics to maintain peptide bioavailability within the dermis, and (4) delivering synergistic skincare benefits. Comprehensive stability assays, in vitro release studies, and percutaneous penetration tests were conducted, demonstrating that BluX Supertide significantly improves GHK-Cu delivery and functional performance compared to unmodified HA systems. These findings establish HA–DPAH supramolecular networks as a versatile and promising platform for the efficient topical delivery of metal-coordinated peptides and other hydrophilic actives.

2. Materials and Methods

2.1. Materials

GHK-Cu peptide was purchased from JYMed Technology Co., Ltd.(Shenzhen, China). Hyaluronic acid (HA) (molecular weight = 0.85~1.6 MDa) was obtained from TS Biotech Co., Ltd.(Shandong, China). Dihydroxypropyl arginine hydrochloride (DPAH) was provided by Seiwa Kasei Co., Ltd. (Osaka, Japan). All other reagents and materials used in this study were obtained from common commercial suppliers and were of analytical grade.

2.2. Preparation and Structural Characterization

BluX Supertide was prepared via a patented microfluidic process, achieving a final GHK-Cu content of 0.67%. A contrast sample, referred to as the GHK-Cu mixture, was prepared using the same components through a simple mixing procedure.

The structural characteristics of BluX Supertide were analyzed by dynamic light scattering (DLS) and cryo-transmission electron microscopy (cryo-TEM) following standard protocols. Synchrotron-based solution small-angle X-ray scattering (SAXS) measurements were conducted at the DND-CAT 5-ID-D beamline of the Advanced Photon Source (Argonne National Laboratory, Argonne, IL, USA), using an X-ray wavelength of 1.37 Å (corresponding to an energy of 9.0 keV), following established beamline experimental and analytical procedures.

2.3. Active Stability Test

The GHK-Cu mixture and BluX Supertide samples were stored under ultraviolet irradiation, 45 °C, 25 °C, and 4 °C conditions for 4 weeks. The GHK-Cu content change in each group was subsequently quantified by high-performance liquid chromatography (HPLC).

2.4. In Vitro Release Test

BluX Supertide and GHK-Cu mixture were separately loaded into individual capped dialysis membranes, which allowed the passage of free active substances but retained the carrier. Each dialysis unit was immersed in deionsed water as the release medium and vertically placed in a thermostatic oscillating incubator. The incubator parameters (temperature, speed and illumination) were set according to the simulated environmental conditions. At predetermined time points, 1 mL of the release medium was withdrawn from each group for concentration analysis, and an equal volume of fresh release medium at the same temperature and pH was replenished to maintain a constant total volume. The concentration of released GHK-Cu was quantified using HPLC method.

2.5. In Vitro Penetration Test

Strat-M® synthetic membranes were used to simulate skin for transdermal testing in a vertical Franz diffusion cell. The intact membrane was fixed between the donor and receptor compartments, with the shiny side facing upward. The receptor compartment was filled with 8 mL of buffer solution, and the system was maintained at a constant temperature of 32 ± 1 °C by circulating water, with continuous stirring at 300 rpm. Subsequently, 1 mL of each sample (BluX Supertide and GHK-Cu mixture) was separately applied to the donor compartment to initiate the transdermal permeation test. At predetermined time points (1, 3, 6, 9, 12, and 24 h), 1 mL of receptor solution was withdrawn for analysis and immediately replaced with an equal volume of fresh, isothermal receptor solution to maintain sink conditions. The cumulative permeation amount of GHK-Cu at each time point was quantified, and a permeation curve was plotted to evaluate and compare the transdermal delivery efficiency of each sample in vitro.

2.5 Cell Scratch Assay

The reparative efficacy of test samples was evaluated using a scratch assay on human umbilical cord mesenchymal stem cells (HUCMSCs), which are multipotent precursor cells derived from Wharton's jelly and perivascular tissue with demonstrated self-renewal and regenerative capacity. After digestion with trypsin, HUCMSCs were centrifuged, resuspended in complete medium, and seeded into 12-well plates at a density of 40,000 cells/cm² (5×10^6 cells/mL). Following 24 h of incubation to allow cell attachment and confluence, scratches were made perpendicularly across the cell monolayer using a sterile 20-μL pipette tip, ensuring uniform scratch width. Detached cells and debris were removed by rinsing with PBS three times. Test samples were applied at a final concentration of 0.5 wt%, and a blank control group was included. Microscopic images were taken immediately after scratching and every 2–4 hours thereafter. The scratch area at each time point was quantified using ImageJ software, and the cell migration rate was calculated to assess wound closure dynamics over time.

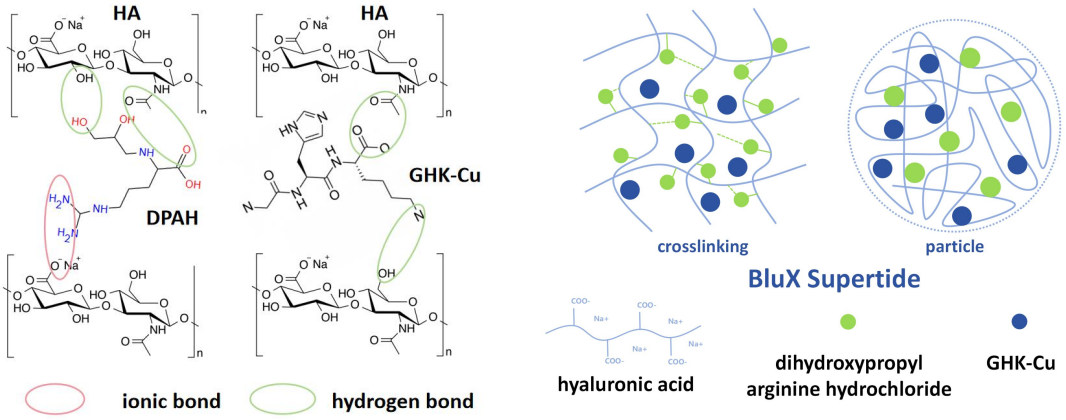
2.6 Suface Filming Characterization by Cross-Polarized Microscopy

To prepare simulated skin substrates, silicon wafers were first cleaned using a mixture of concentrated sulfuric acid (H_2SO_4), hydrogen peroxide (H_2O_2), and deionized water in a 1:1:1 ratio. The wafers were placed on a hotplate at 110 °C for 1 hour and subsequently rinsed thoroughly with deionized water. This cleaning procedure generated a highly hydrophilic surface due to the formation of a thin natural oxide layer (SiOx). To produce a hydrophobic surface, the wafers were further immersed in aqueous hydrogen fluoride (HF) solution for 30 seconds to remove the SiOx layer, yielding a hydrogen-terminated silicon surface. For surface filming analysis, 100 µL of each sample was spin-coated onto the treated silicon substrates and dried at 25 °C. The resulting surface films were then evaluated by cross-polarized optical microscopy to observe film uniformity and birefringent structural features.

3. Results

3.1 Characteristics of BluX Supertide

We propose that BluX Supertide is formed through a supramolecular self-assembly process driven by ionic interactions and hydrogen bonding among HA, DPAH, and GHK-Cu, as illustrated schematically in Figure 1.



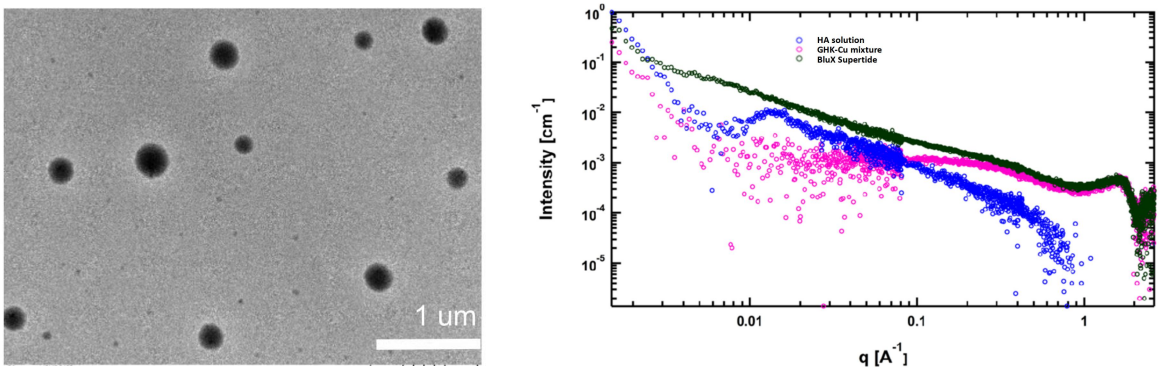
(**a**) (**b**)

Figure 1. The structural schematic diagram of BluX Supertide. (a) Interaction between HA, DPAH and GHK-Cu. (b) Particles formation of BluX Supertide.

As shown in Figure 2a, cryo-transmission electron microscopy (cryo-TEM) revealed that BluX Supertide exhibits a well-defined spherical morphology with particle diameters ranging from 100 to 300 nm. This structural feature is consistent with the dynamic light scattering (DLS) data, which showed an average hydrodynamic diameter of approximately 271 nm, indicating successful encapsulation of GHK-Cu within the nanostructures.

Further insights were provided by small-angle X-ray scattering (SAXS) analysis (Figure 2b). BluX Supertide (green line) displayed a smooth, continuous scattering profile across the q-range, characteristic of stable and uniform nanostructure formation. In contrast, the GHK-Cu mixture (pink line) exhibited weak and irregular scattering signals, indicative of disordered or unassembled components. The HA solution alone (blue line) showed a pronounced interaction peak in the mid-q region, attributed to strong electrostatic interactions among HA chains. Notably, this peak was entirely smeared out in the BluX Supertide curve, supporting the hypothesis that the supramolecular assembly of HA with DPAH and GHK-Cu

disrupts native HA interactions and is primarily driven by ionic coordination and hydrogen bonding.



(**a**) (**b**)

Figure 2. Structural characterization of BluX Supertide. (a) cryo-TEM image showing the spherical morphology of BluX Supertide nanoparticles. (b) SAXS intensity profiles of HA solution (blue), GHK-Cu mixture (pink), and BluX Supertide (green), illustrating the formation of organized supramolecular structures in BluX Supertide.

3.2 Stability test

As shown in Figure 3, GHK-Cu exhibited progressive degradation over time, which may compromise its functional efficacy. In contrast, BluX Supertide effectively preserved GHK-Cu content under various storage conditions for up to 4 weeks, maintaining more than 85% to 95% of the initial concentration. This was consistently higher than the free GHK-Cu mixture under identical conditions. These findings demonstrate that the HA–DPAH supramolecular carrier significantly enhances the chemical stability of GHK-Cu, likely by shielding the labile copper–peptide coordination from environmental degradation.

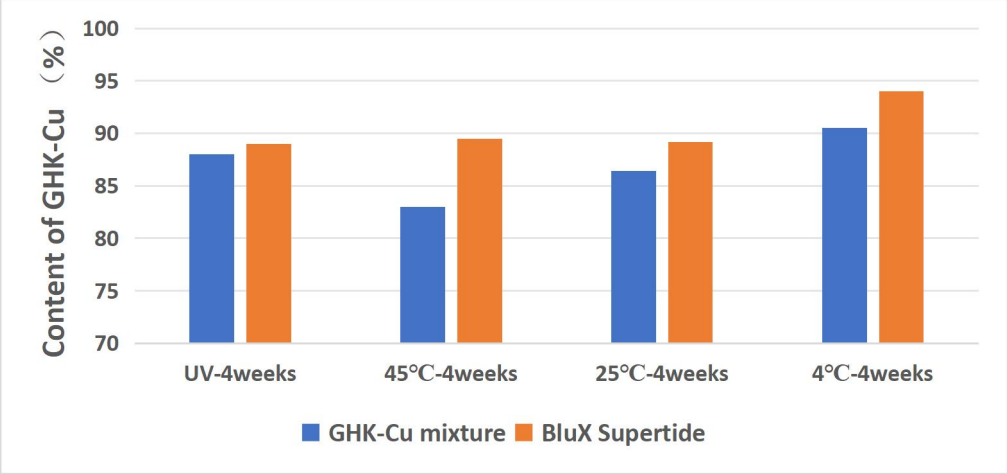


Figure 3. Stability of remaining GHK-Cu content (%) under various storage conditions (UV exposure, 45°C, 25°C, and 4°C) over 4 weeks.

3.3 In Vitro Release test

As shown in Figure 4, the GHK-Cu mixture exhibited a rapid release profile, reaching near-complete release in a short period, indicating the absence of a controlled-release effect. In contrast, BluX Supertide demonstrated a sustained release behavior, with GHK-Cu maintaining a steady release level of approximately 60% over a 24-hour period. This result highlights the superior controlled-release capability of the BluX Supertide system, attributed to the supramolecular encapsulation and gradual diffusion of GHK-Cu from the HA–DPAH matrix.

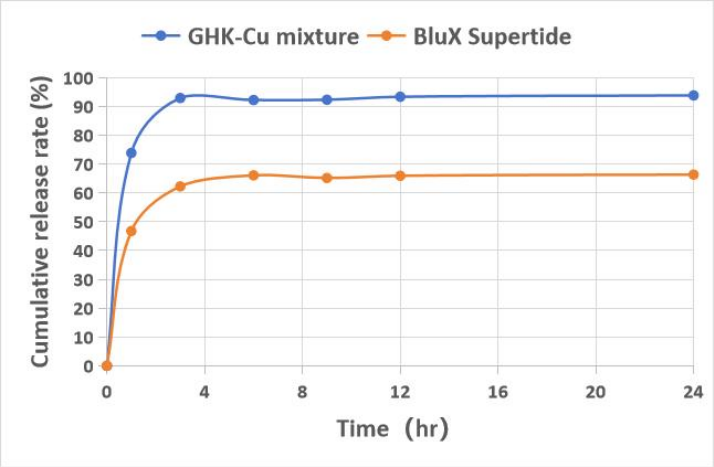


Figure 4. In vitro release profiles of GHK-Cu mixture and BluX Supertide over 24 hours.

3.4 In Vitro Penetration test

As shown in Figure 5, BluX Supertide demonstrated faster and more efficient penetration of GHK-Cu compared to the GHK-Cu mixture. At the 6-hour time point, the penetration amount of GHK-Cu in BluX Supertide approached the maximum level observed for the GHK-Cu mixture, exhibiting a 183% increase in penetration. This result confirms that BluX Supertide significantly enhances transdermal delivery compared to the simple GHK-Cu mixture.

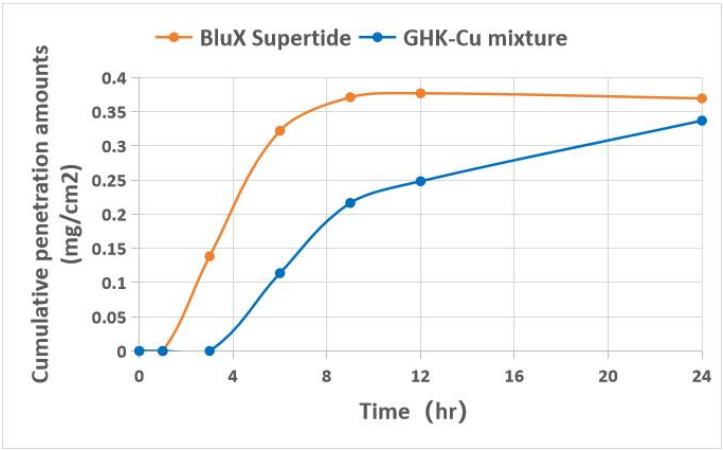


Figure 5. In vitro transdermal penetration profiles of GHK-Cu mixture and BluX Supertide over 24 hours.

3.5 Reparative efficacy

As shown in Figure 6, the GHK-Cu mixture group demonstrated significant reparative efficacy on cell migration compared to the blank group. However, the BluX Supertide group exhibited even higher reparative efficiency. At the 3-hour time point, BluX Supertide enhanced stem cell repair by 460% compared to the controls and by 43% compared to the GHK-Cu mixture. This improvement can be attributed to the stable crosslinked structure of HA and DPAH, along with the successful encapsulation of GHK-Cu, which together enhance the bioavailability and therapeutic efficacy of GHK-Cu.

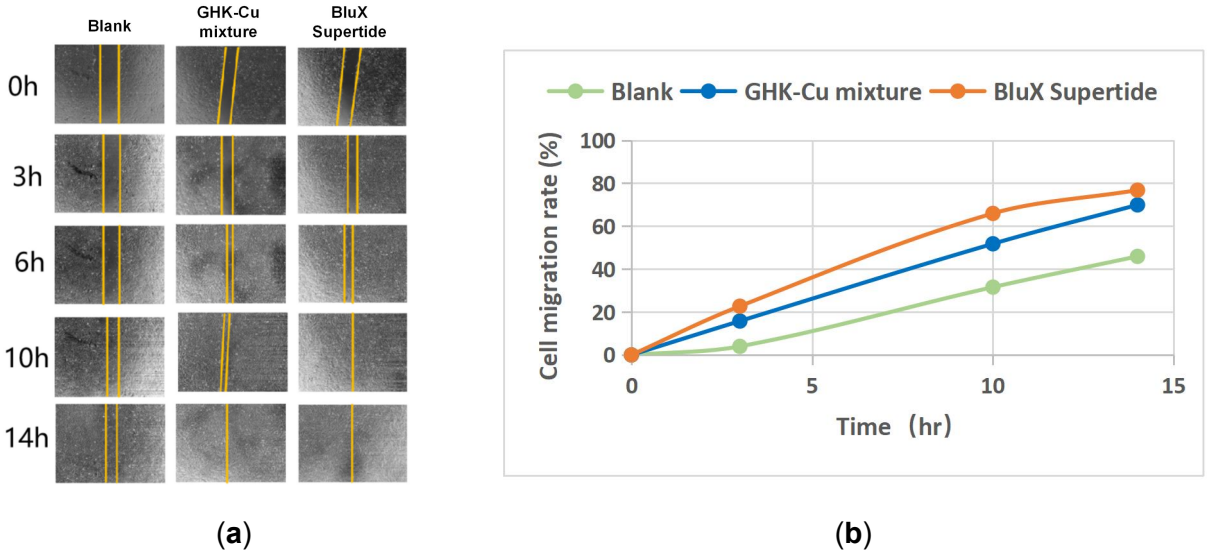


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Figure 6. Reparative efficacy assessed by cell scratch assay. (a) Representative images showing cell migration in blank, GHK-Cu mixture, and BluX Supertide groups. (b) Cell migration rate curves for blank, GHK-Cu mixture, and BluX Supertide groups, illustrating the enhanced reparative efficacy of BluX Supertide.

3.6 Suface Filming Property

As shown in Figure 7, the GHK-Cu mixture residue was scarcely observed on the wafer, indicating poor filming ability and no surface filming structures on a hydrophobic surface. In contrast, the microscopic image of BluX Supertide revealed that the sample formed a regular liquid-crystalline structure, fully adhering to the surface of the hydrophobic wafer. This unique surface filming structure suggests its potential as a skin adhesive, which could significantly enhance its topical efficacy. Moreover, this distinctive structure may contribute to improved skin compatibility and overall skin benefits, warranting further investigation into its underlying mechanisms and long-term effects on skin health.

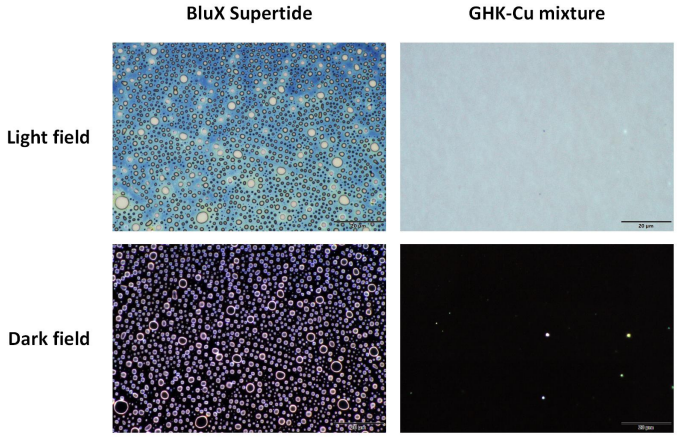

Figure 7. Microscopic images of BluX Supertide and GHK-Cu mixture on a hydrophobic wafer. The image highlights the distinct surface filming properties of BluX Supertide, which forms a regular liquid-crystalline structure adhering to the hydrophobic wafer, compared to the sparse residue of the GHK-Cu mixture.

4. Discussion

HA is an ideal polymer for the development of advanced delivery systems due to its linear structure and the abundance of functional groups, including carboxylate, hydroxyl, and acetamido groups. These features enable versatile crosslinking strategies, making HA suitable for various applications [15]. In this study, we innovatively created and systematically characterized BluX Supertide, a delivery system formed by crosslinking HA with DPAH through electrostatic interactions and hydrogen bonds. DPAH acts as a small molecular "linker," carrying both positive charges and hydroxyl groups that facilitate the physical crosslinking of HA, resulting in a stable carrier complex. GHK-Cu is encapsulated within this structure through hydrogen bonding between HA and DPAH. The use of physically dynamic crosslinking for HA offers superior quality and safety control compared to chemical crosslinking methods.

In addition to serving as an effective delivery system, both HA and DPAH provide nourishment to the skin, creating a synergistic microenvironment that promotes cellular hydration and overall skin health [16,17]. The HA-based structure is soft and flexible, allowing for the efficient encapsulation of GHK-Cu, preventing its inactivation by competing components, and ensuring effective transdermal penetration. Furthermore, the system exhibits remarkable adhesive properties on hydrophobic surfaces, highlighting its potential for enhancing topical efficacy. Thus, we propose that this HA-based delivery system is highly suitable for GHK-Cu, especially in skin regeneration applications.

The effectiveness of the HA-based system in enhancing GHK-Cu's stability, delivery, and regenerative function has been validated through various assessments. Beyond GHK-Cu, we hypothesize that BluX Supertide could be innovatively utilized as a co-delivery system for other peptides. Many peptides face challenges related to poor bioavailability and compatibility,

yet they contain functional groups conducive to molecular interactions, enabling successful encapsulation [18]. Moving forward, we envision the co-encapsulation of multiple anti-aging peptides within BluX Supertide, which could synergistically amplify their regenerative effects, paving the way for innovative treatments in anti-aging skincare.

5. Conclusion

To overcome the limitations of GHK-Cu and enhance its efficacy, we introduced a novel delivery system, BluX Supertide, which is based on the physical crosslinking of HA and DPAH. We have confirmed that this system forms a stable spherical structure, ideal for use as a carrier. The excellent biocompatibility and moisturizing properties of HA and DPAH enable effective encapsulation of hydrophilic GHK-Cu, thus improving its stability, release, delivery, and reparative efficacy. BluX Supertide offers a multifaceted approach to dermal anti-aging treatments, opening up new possibilities for both skincare and regenerative medicine. By enhancing the effectiveness of GHK-Cu and other similar actives, BluX Supertide holds the potential to significantly advance topical drug delivery, providing valuable benefits for therapeutic and cosmetic applications aimed at addressing age-related skin concerns.

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