A Deep Eutectic Solvent Bioadhesive Based on a Mixture of Peptides from *Periplaneta americana* and Snake Slough

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***Abstract*—The repair of surgical wounds and tissue defects remains a significant clinical challenge, often necessitating advanced wound management strategies. Inspired by the traditional use of *Periplaneta americana* and snake slough in wound healing, this study developed a novel bioadhesive by integrating their bioactive peptide extracts within a deep eutectic solvent framework. The three components form a homogeneous composite system through strong eutectic effects and self-assembly, solidifying at the affected area via a simple preheating-cooling method. The deep eutectic solvent serves multiple functions as a co-solvent, plasticizer, penetration enhancer, and antibacterial agent. The preparation process is rapid, mild, and scalable, utilizing green raw materials with no additional additives. The resulting bioadhesive exhibited a temperature-induced sol-gel transition, enabling convenient application and in situ solidification. Due to the presence of hydrogen bonding, electrostatic interactions, and van der Waals forces, the prepared bioadhesive exhibited excellent adhesive properties. In vitro release studies demonstrated a sustained polypeptide release profile. Stability assessment over 90 days at room temperature showed no significant changes in polypeptide content, appearance, or odor, owing to the stabilizing and antimicrobial properties of the deep eutectic solvent. This multifunctional bioadhesive, leveraging the synergistic therapeutic effects of its components, shows great potential for surgical repair and wound healing applications.**

***Keywords*—deep eutectic solvent, bioadhesive, two animal peptides, multifunctional material, wound healing**

I. Introduction

Periplaneta americana is a traditional Chinese medicinal insect whose extracts are rich in small-molecule peptides, amino acids, and various bioactive compounds, exhibiting significant tissue repair-promoting activity [1]. Both fundamental research and clinical applications have confirmed that *Periplaneta americana* extracts, notably represented by the commercial product Kangfuxin Liquid, demonstrate excellent therapeutic efficacy in treating burns, traumatic injuries, ulcers, and post-surgical wound repair [2]. However, with the continuous increase in production capacity of related pharmaceuticals, the volume of alcohol-extracted waste residue generated from *Periplaneta americana* has risen substantially year by year. This residual material still retains a considerable amount of bioactive substances, including proteins and peptides, presenting substantial potential for redevelopment and valorization [3]. Unfortunately, this residue is typically discarded as waste, leading not only to a squandering of valuable bioresources but also to certain environmental pressures due to disposal challenges. From a pharmacological perspective, the peptides, polysaccharides, and other compounds remaining in the *Periplaneta americana* waste residue have been verified to retain multiple beneficial bioactivities, including anti-inflammatory, antibacterial, and antioxidant effects. Furthermore, they continue to promote cell proliferation, granulation tissue formation, and overall wound healing [4]. These inherent properties lay a solid foundation for the clinical development of novel therapeutic materials aimed at enhancing tissue regeneration and wound repair.

Snake slough, the dried epidermal membrane naturally shed by snakes of the family Colubridae, is primarily composed of keratin, collagen, amino acids, fatty acids, sterols, and other constituents. Clinical studies have demonstrated its notable therapeutic efficacy in treating herpes zoster, where it promotes the crusting and resolution of herpetic lesions while alleviating associated pain. Furthermore, snake slough is frequently incorporated into compound external powder formulations for trauma treatment, exhibiting analgesic and hemostatic properties while facilitating wound crusting and healing. Modern pharmacological research has confirmed that extracts derived from snake slough contain bioactive components with antibacterial, anti-inflammatory, and wound healing-promoting activities [5]. Notably, when snake slough keratin undergoes appropriate hydrolysis, it yields polypeptide fragments with specific structures and functions capable of promoting fibroblast proliferation and epidermal regeneration, thereby accelerating wound healing and demonstrating exceptional medicinal value [6]. In recent years, researchers have increasingly

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focused on the hydrolyzed products of snake slough keratin, which possess distinct advantages in external preparations such as wound dressings and tissue adhesives [7]. Additionally, these macromolecules can self-assemble into β-sheet secondary structures, endowing the materials with favorable film-forming properties and mechanical stability.

Deep Eutectic Solvents (DESs) are eutectic mixtures formed through hydrogen bonding interactions between hydrogen bond acceptors and hydrogen bond donors at specific stoichiometric ratios, exhibiting melting points significantly lower than those of their individual components [8]. As a novel class of green solvents, DESs offer advantages including simple preparation, low cost, excellent biocompatibility, and biodegradability, demonstrating broad application prospects in areas such as drug solubilization, transdermal absorption enhancement, and biomaterial fabrication. In the field of medical materials, research on the application of DESs has grown increasingly sophisticated [9]. As pharmaceutical excipients, DESs can be employed to improve drug solubility and transdermal permeability. In biomaterial preparation, DESs serve as green processing media for pharmaceutical excipients such as microcrystalline cellulose, yielding materials with favorable release characteristics. Furthermore, certain DESs themselves possess inherent antibacterial activity, enabling them to function as multifunctional excipients in biomedical materials, playing multiple roles including plasticization, permeation enhancement, and bacteriostasis [10].

Bioadhesives refer to a class of materials capable of forming adhesion with biological tissues. Their ease of use enables rapid tissue sealing and hemostasis while minimizing secondary damage to patients, garnering significant clinical attention as an alternative to traditional suture techniques [11]. Existing bioadhesives are primarily categorized into two major classes: natural bioadhesives and synthetic bioadhesives. Natural bioadhesives, such as fibrin glue and gelatin-based adhesives, exhibit excellent biocompatibility with mild inflammatory and foreign body responses. However, they commonly suffer from limitations including low adhesion strength under wet conditions, inconsistent quality, and poor thermal stability [12]. Additionally, fibrin glue derived from blood products raises biosafety concerns and entails high costs, restricting its widespread application. Synthetic bioadhesives, including cyanoacrylates, Polyethylene Glycol (PEG)-based adhesives, and polyurethane adhesives, offer advantages such as high adhesion strength, rapid curing, and stable performance [13]. Nevertheless, these materials often demonstrate poor biocompatibility, potentially inducing severe inflammatory responses and immune rejection. Their degradation products may exhibit cytotoxicity, and the exothermic polymerization process can cause thermal damage to surrounding tissues. Although synthetic polymer bioadhesives possess certain advantages in mechanical properties and tunability, their deficiencies in wet adhesion, biocompatibility, and mechanical matching remain insufficient to meet clinical requirements. Achieving the simultaneous optimization of excellent biocompatibility, appropriate mechanical strength, controlled degradability, and biological functionality that promotes tissue repair continues to pose a significant challenge in bioadhesive research and development [14]. Therefore, developing a novel bioadhesive derived from natural sources that combines pro-regenerative activity with favorable mechanical properties and enables controlled solidification through simple methods holds substantial clinical significance and application value. In this context, this study aims to prepare a deep eutectic solvent bioadhesive based on a mixture of peptides from *Periplaneta americana* and snake slough. This design synergistically combines the tissue-repairing activity of the two animal-derived peptides with the multifunctional properties of deep eutectic solvents (plasticizing, permeation-enhancing, and antibacterial). The resulting adhesive achieves rapid gelation via a simple preheating-cooling process, offering a green and scalable strategy to address the biocompatibility and mechanical limitations of current bioadhesives for surgical applications.

II. Preparation of Deep Eutectic Solvent Bioadhesive

A. Preparation of Periplaneta americana polypeptide Extracts

The *Periplaneta americana* polypeptide extract was prepared using a multi-step procedure involving ultrasonic-assisted extraction, ethanol precipitation, dialysis, and lyophilization. Initially, 10 g of defatted *Periplaneta americana* (Fig. 1a) dry powder (120 mesh) was immersed in 150 mL of 75% ethanol and subjected to ultrasonic extraction at 300 W for 30 minutes to facilitate the release of intracellular bioactive components. After filtration, the residual biomass was re-extracted under identical conditions to maximize yield, and the two extracts were combined, resulting in a transparent orange-yellow solution. The combined extract was then concentrated by rotary evaporation under reduced pressure until the ethanol was completely removed. To precipitate the crude polypeptide fraction, three volumes of 95% ethanol were added to the concentrated aqueous solution, and the mixture was allowed to stand at 4°C for 12 hours to ensure complete precipitation. The resulting precipitate was collected by centrifugation at 4000 rpm for 5 minutes, redissolved in Tris-HCl buffer solution under stirring, and subsequently subjected to dialysis using a semi-permeable membrane with a Molecular Weight Cut-Off (MWCO) of 1000 Da. This dialysis step effectively removed low-molecular-weight impurities while retaining the target polypeptide fraction. Finally, the dialysate (external solution) was collected, concentrated under reduced pressure, and lyophilized to obtain the purified *Periplaneta americana* polypeptide extract as a dry powder for subsequent experiments. The *Periplaneta americana* polypeptide extract was obtained as a light yellow amorphous powder, as shown in Fig. 2a.



Fig. 1. The raw materials of (a) *Periplaneta americana* and (b) snake slough in the China market.

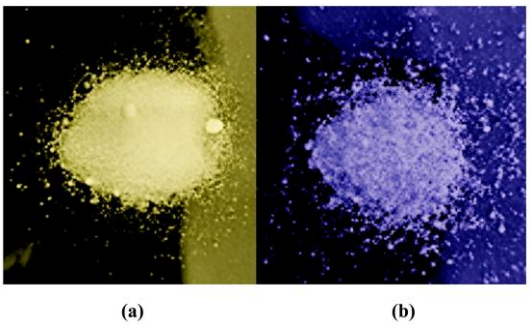


Fig. 2. Photographs of (a) the *Periplaneta americana* polypeptide extract and (b) the snake slough polypeptide extract.

B. Preparation of Snake Slough Polypeptide Extracts

At present, there are three snake medicinal herbs included in the Chinese Pharmacopoeia: Agkistrodon acutus, Bungarus parvus, and Ptyas dhumnades. The three kinds of snake slough have similar main components and can all be used to prepare this biological adhesive. 10 g of snake slough (Fig. 1b) dry powder (100 mesh) was immersed in 200 mL of 60% ethanol and extracted at 50°C for 2 h to remove pigments and impurities. After extraction, the mixture was filtered while hot, and the residue was washed three times with water. The resulting residue was air-dried, then ultrapure water (solid-to-liquid ratio of 1:3 g/mL) and 2500 U/g of immobilized alkaline protease were added. Enzymatic hydrolysis was performed at 42°C for 4 h to release bioactive peptides. Subsequently, 10 volumes of ultrapure water were added to the hydrolysate, and the mixture was extracted at 50°C for 2 h to maximize peptide recovery. After extraction, the mixture was filtered while hot. Finally, the filtrate was dialyzed using a semi-permeable membrane with a molecular weight cut-off of 2000 Da to remove salts and low-molecular-weight impurities. The retained solution was collected, concentrated under reduced pressure, and lyophilized to obtain the purified snake slough polypeptide extract. The obtained snake slough polypeptide extract appeared as beige amorphous granules, as shown in Fig. 2b.

C. Preparation of Deep Eutectic Bioadhesive Composite

The bioactive composite system was prepared by combining *Periplaneta americana* and snake slough polypeptides at a mass ratio of 3:7 (g/g). This polypeptide mixture was then incorporated into a deep eutectic solvent composed of betaine and phytic acid at a molar ratio of 3:1. The blending process was conducted at a solid-to-liquid ratio of 1:3 (g/mL) by dispersing the polypeptides into the deep eutectic solvent using ultrasonic-assisted treatment at 300 W and 55°C for 10 min. This step ensured thorough mixing and facilitated hydrogen bonding interactions between the polypeptide components and the deep eutectic solvent matrix, resulting in the formation of a homogeneous deep eutectic bioadhesive composite system. The obtained formulation was subsequently sterilized (by ultraviolet irradiation for 30 min) and sealed for further use.

The sol-gel transition behavior of the as-prepared deep eutectic bioadhesive was evaluated through a controlled thermal treatment process. The formulation was initially preheated to 60°C in a water bath to ensure complete fluidity and homogeneity. Upon gradual cooling to near room temperature, the system underwent preliminary gelation. Subsequent rapid cooling induced complete solidification, resulting in the formation of a stable three-dimensional network structure. A comparative visual depiction of the bioadhesive before and after this thermally induced phase transition is presented in Fig. 3.

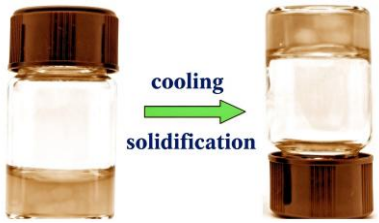


Fig. 3. Sol-gel transition of the DES bioadhesive.

III. Temperature-Induced Polypeptide Particle Size Variation in the Deep Eutectic Solvent Bioadhesive

The self-assembly behavior of polypeptides within the deep eutectic bioadhesive system was investigated by measuring their average particle size under three different thermal treatment conditions. Dynamic Light Scattering (DLS) was employed to determine the average hydrodynamic diameter of polypeptides in samples subjected to distinct temperature protocols: (i) after preheating to 60°C; (ii) after gradual cooling to near room temperature (fluid state); and (iii) after rapid cooling-induced solidification.

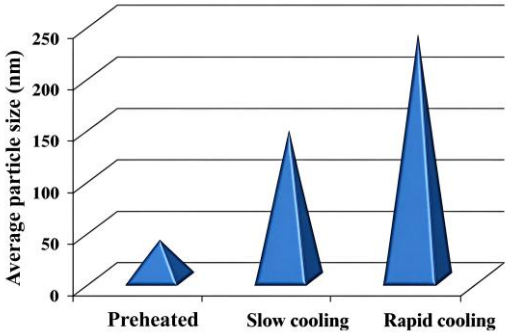


Fig. 4. Average particle size of the deep eutectic bioadhesive subjected to distinct thermal treatments.

As shown in Fig. 4, the average particle size of polypeptides exhibited a progressive increase across these three conditions. This size evolution indicates the occurrence of temperature-induced self-assembly, wherein polypeptides gradually aggregate into larger supramolecular structures depending on the thermal history applied. The gradual increase in polypeptide particle size under different thermal protocols demonstrates a unique thermally triggered self-assembly mechanism facilitated by the deep eutectic solvent. Unlike conventional aqueous systems, this DES-mediated assembly enables controlled polypeptide aggregation through tunable hydrogen-bonding interactions.

IV. Adhesive Strength of the Deep Eutectic Bioadhesive

Four deep eutectic solvent-based bioadhesive composite systems were prepared. *Periplaneta americana* and snake slough peptides were mixed at a mass ratio of 3:7 (g/g), then blended with different Deep Eutectic Solvents (DES) at various mass-to-volume ratios and sonicated under different conditions: System 1 employed betaine–phytic acid DES (molar ratio 3:1) at a mass-to-volume ratio of 1:3 g/mL with ultrasonication at 55°C and 300 W for 10 min; System 2 used betaine–lactic acid DES (molar ratio 1:1) at 1:3 g/mL under the same sonication conditions; System 3 adopted betaine–phytic acid DES (molar ratio 3:1) at 1:5 g/mL with ultrasonication at 60°C and 300 W for 20 min; and System 4 utilized betaine–lactic acid DES (molar ratio 1:1) at 1:5 g/mL under the same sonication parameters as System 3.

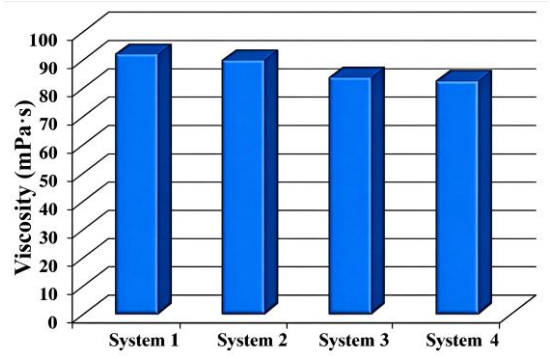


Fig. 5. The adhesive strength of bioadhesives prepared with different DESs.

The adhesive strength of bioadhesive composite systems was evaluated, as shown in Fig. 5. Two pieces of Thermoplastic Elastomer (TPE/TPR) synthetic rubber (1 cm × 3 cm, 5 g each) were used to simulate human tissue, with one piece attached to the balance tray and the other fixed onto the balance base. The preheated and gradually cooled bioadhesive was applied to the contact surfaces of both rubber pieces. After bringing them into contact, the assembly was rapidly cooled and compressed with a 50 g weight for 10 min. Subsequently, weights were progressively added to the other tray until the two rubber pieces detached within 5 s, and the total weight added was recorded as the adhesive strength. The bioadhesive (system 1) demonstrated the highest adhesive strength among all tested formulations. Based on the chemical structures of the components, the proposed adhesion mechanisms primarily involve hydrogen bonding, electrostatic interactions, and van der Waals forces. Although TPE/TPR synthetic rubber serves as a useful initial screening platform, future validation of adhesive strength on biological tissues such as porcine skin is necessary to better approximate the in vivo environment and support translational feasibility.

V. In Vitro Polypeptide Release from the Deep Eutectic Bioadhesive System

The in vitro release behavior of polypeptides from the deep eutectic bioadhesive prepared in Example 3 was investigated, as shown in Fig. 6. A PDMS medical membrane (0.5 mm thickness, 1.5 cm × 1.5 cm area) was selected to simulate human skin, and the deep eutectic bioadhesive was tested in its solidified state. At predetermined time intervals, 1 mL of sample was accurately withdrawn from the sampling port, and an equal volume (1 mL) of normal saline was immediately added to the receiver compartment to maintain constant volume. Each experiment was performed in triplicate. The cumulative release amount per unit area (mg/cm²) was calculated based on the polypeptide concentration (mg/mL) in the collected samples. Polypeptide quantification was carried out using the Folin–Ciocalteu method (Lowry assay). The in vitro release results indicated that the deep eutectic bioadhesive exhibited a sustained release profile of the functional polypeptides, which is favorable for prolonging their therapeutic effects at the application site.

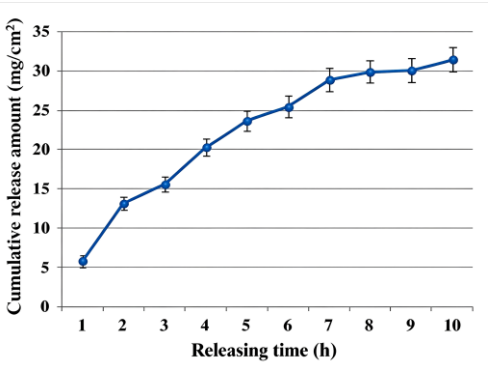


Fig. 6. In vitro release behavior of polypeptides from the deep eutectic bioadhesive.

VI. Stability of the Deep Eutectic Bioadhesive

Considering the potential instability of polypeptide components, the stability of the deep eutectic bioadhesive stored sealed at room temperature (20–35°C) was evaluated based on the trend of polypeptide content variation. Owing to the stabilizing effect and antimicrobial preservative properties of the deep eutectic solvent, no additional components were required. As shown in Fig. 7, no significant decrease in polypeptide content was observed over 90 days, and no substantial changes in

overall appearance, odor, or color were detected. These results fully demonstrate that the developed system possesses good stability.

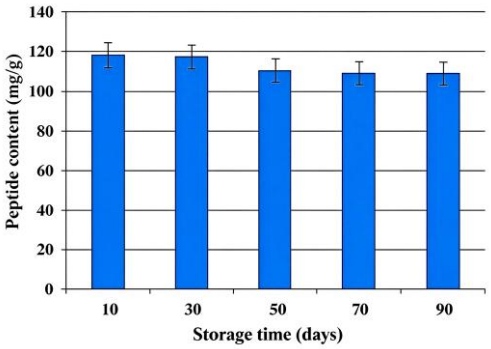


Fig. 7. The stability of deep eutectic bioadhesives.

VII. Biocompatibility of DES

The widely acknowledged zebrafish model was employed to assess the safety and biocompatibility of green solvents. Since the experiment necessitated a solvent with no biological toxicity, the applied DES was chosen and subjected to a toxicity test in accordance with Chinese national standard GB/T 13267-1991. All the zebrafish survived throughout the 96-hour experiment, demonstrating the very low biotoxicity of this DES. Due to the use of edible natural components, the risk of using the DES is greatly reduced, which is important for this kind of biomedical medium.

VIII. Conclusion

A novel bioadhesive was successfully constructed by integrating *Periplaneta americana* and snake slough polypeptides into deep eutectic solvent systems. The bioadhesive exhibited temperature-induced sol-gel transition, enabling convenient application and in situ solidification. Polypeptide self-assembly during thermal treatment was confirmed by particle size analysis. Due to the presence of hydrogen bonding, electrostatic interactions, and van der Waals forces, the prepared bioadhesive exhibited excellent adhesive properties. In vitro release studies demonstrated a sustained polypeptide release profile. Stability assessment over 90 days at room temperature showed no significant changes in polypeptide content, appearance, or odor, owing to the stabilizing and antimicrobial properties of the deep eutectic solvent. This multifunctional bioadhesive shows great potential for surgical repair and wound healing applications.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Zidan Luo conducted the research and wrote the paper; Ruirui Li analyzed the data; Shun Yao supervised this paper; all authors approved the final version.

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