Egg Albumen as a Fast and Strong Medical Adhesive Glue

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Sutures penetrate tissues to close wounds. This process leads to inflammatory responses, prolongs healing time, and increases operation complexity. It becomes even worse when sutures are applied to stress-sensitive and fragile tissues. By bonding tissues via forming covalent bonds, some medical adhesives are not convenient to be used by surgeons and have side effects to the tissues. Here egg albumen adhesive (EAA) is reported with ultrahigh adhesive strength to bond various types of materials and can be easily used without any chemical and physical modifications. Compared with several commercial medical glues, EAA exhibits stronger adhesive property on porcine skin, glass, polydimethylsiloxane. The EAA also shows exceptional underwater adhesive strength. Finally, wound closure using EAA on poly(caprolactone) nanofibrous sheet and general sutures is investigated and compared in a rat wound model. EAA also does not show strong long-term inflammatory response, suggesting that EAA has potential as a medical glue, considering its abundant source, simple fabrication process, inherent nontoxicity, and low cost.

1. Introduction

As an important component in surgical treatments, incision closure techniques attracted increased attentions in clinic study due to its critical role in wound healing and recovery in recent years.[1] Aiming to decrease operation time, accelerate recovery and alleviate patients’ pain, state of the art wound closure techniques requires a new generation of materials from synthetic and bioabsorbable sutures[2] to surgical staples and bioadhesive glues.[3] Despite various forms of sutures already on bedside, there were similar drawbacks, such as inflammatory responses, scar tissues, second injury of tissues, and prolonged healing time.[4] Adhesive glue could provide a facile way to close wound within less operation time via a minimum invasion, which can avoid further wound injury and lead to a better appearance after recovery.[5]

Currently, those reported bioadhesives are thrown into the following classifications: natural protein derived—fibrin,[6] albumin,[7] gelatin,[8] collagen,[9] polysaccharide—chitosan,[10] alginate,[11] hyaluronic acid,[12] dextran,[13] synthetic polymers—polycyanoacrylate,[14] polyurethane,[15] polyethylene glycol,[16] and biomimetic mussel-inspired dopamine,[17] etc. The ultimate goals for ideal bioadhesives are strong adhesion strength, low cost, good biocompatibility, and facile procedure. While current common bioadhesives either exhibit low adhesion strength capacity (such as fibrin gel, gelatin, collagen) and require extra crosslinker reagents for wound closure (e.g., glutaraldehyde,[18] N-hydroxy succinimide ester,[19] thiol,[19] photo-crosslinker),[20] or have good adhesion strength at the cost of noticeable toxicity (e.g., cyanoacrylate).[21] As a most used commercial synthetic adhesives, cyanoacrylate and its derivatives have excellent sealing performance owing to its moisture-initiated fast polymerization,[22] however, their toxicity and potential toxicity of their degradation products are major concerns in their applications.[23] Although fibrin based biological glues have good biocompatibility, their applications are limited since their adhesive strengths are not reliable.[24] Inspired by mussel's strong adhesion to rocks in complex ocean underwater environments, dopamine based glues were discovered and gained enormous attentions.[17a,18,25,26] In our previous work, dopamine-based crosslinker-conjugated gelatin/poly(caprolactone) nanofibrous sheets exhibited good adhesion performance for suture-free incision closure.[26] However, the requirements of presence of the chemical oxidants, such as FeCl₃, HClO₄, H₂O₂, or base buffer, and the long polymerization time limited the clinic applications of dopamine-based adhesives.[27]
As one of the most favorable food in nature, egg albumen is inherently nontoxic, biocompatible, biodegradable to human body, and has abundant resources in nature with extra low cost. However, to the best of our knowledge, egg albumen has not been well investigated for medical bioadhesive applications. Here we report egg albumen as biological adhesive with outstanding adhesion capacity via a simple process. Compared with commonly used commercial medical adhesives, egg albumen adhesive (EAA) exhibited a better adhesion strength on pigskin, polydimethylsiloxane (PDMS), and glass substrates, and good underwater performance as well. Then, EAA was coated onto polycaprolactone (PCL) nanofibrous mesh as wound closure patches and promoted wound healing, suggesting EAA’s potential as a medical bioadhesive candidate (Scheme 1). To this end, our finding in egg albumen has its unique values: exceptional adhesion strength, maintaining underwater adhesion, abundant natural resources at low cost (only a few cents per gram), good biocompatibility (no significant inflammatory response and bioabsorbable property), and facile processability.

2. Results and Discussion

Preparation of dry albumen powder was simple and straightforward via grinding slowly air-dried protein aggregates into fine powder in a mortar. Due to the slowly air-drying, the albumen proteins formed highly aggregated morphology, which was rigid and could not completely recovered to homogenous solution status (Figure S1, Supporting Information). Dried albumen powder could form highly sticky gel when mixed with small portion of water, which showed excellent adhesion performance. The gel was then used as an EAA. The optical microscope photograph of EAA was given in Figure S2 (Supporting Information), showing the gel-like appearance of EAA rather than homogenous egg white albumen liquid. The glass slides glued by EAA (25 mm × 20 mm) can sustain a weight of 6 kg, and it could be coated on PCL nanofibrous mesh patch to cure wounds efficiently, as shown in Scheme 1. The glue was prepared at 3 different concentrations, 1.400, 0.875, and 0.636 g powder per mL water. The EAA at concentration of 0.875 g powder per mL water showed the best injectability from a syringe needle (gauge 19, inner diameter of needle is 0.7 mm) with great formability and adhesive ability (Figure 1A–C; Movie S1, Supporting Information), and 0.875 g powder per mL water EAA was chosen for further experiments. Figure 1D stated the complex viscosity (Pa s) versus angular frequency (rad s⁻¹) of EAA. It can be observed that the viscosity decreases with increasing shear rate, indicating a typical shear-thinning behavior. Figure 1E provided the SEM image of EAA coated on PCL nanofibrous membrane, in which albumen powders were mixed and integrated together to form a gel.

The lapse shear adhesion of EAA was conducted on different substrates, including PDMS, glass, and pigskin, and was compared with two commercial available medical adhesives, cyanoacrylate synthetic glue and fibrin glue. EAA exhibited huge differences on different substrates. On hydrophobic PDMS substrates, the shear adhesion of EAA could reach 5.3 ± 1.1 kPa, whereas cyanoacrylate and fibrin glues only had the adhesion strength of 1.8 ± 0.4 and 0.5 ± 0.2 kPa, respectively, as shown in Figure 2A. According to Figure 2B, EAA had a remarkable shear adhesion capacity on hydrophilic glass substrates, which could be 216 ± 80.4 kPa, whereas the shear adhesion was less than 3.0 kPa for the rest two commercial glues. On pigskin tissue (Figure 2C), EAA also exhibited excellent adhesion performance, a high adhesion of 56.2 ± 15.2 kPa, which could be comparable with that of cyanoacrylate synthetic adhesive (55.4 ± 16.9 kPa), and obviously higher than fibrin bioadhesive’s adhesion (24.0 ± 9.3 kPa). Given the extra low cost and environment friendly processing steps of EAA, EAA owns brilliant perspective as a candidate of medical adhesives.

The adhesion capacity of EAA on glass substrates could be further evaluated, as shown in Figure 3A. EAA glue exhibited incredibly strong shear adhesion on glass substrates, and a small adhesion area of 25 mm × 20 mm could afford a weight of 6 kg steadily (Figure 3A; Movie S2, Supporting Information). The calculated shear adhesion strength of EAA on glass was 117.7 kPa, which was outstanding among previously reported...
adhesive materials for glass substrates. For example, Yuk et al. developed an double-network hydrogel with exceptional adhesion property to bond two glass plates together, which could hold a 25 kg object in a adhesion area of 50 mm × 50 mm (a shear stress of 98.1 kPa).[29] Comparatively, our EAA glue displayed a higher adhesion capacity and could be achieved at extra low cost. The EAA was also applied to fix broken bones and exhibited interesting adhesion performance as well. The fractured bone with a cross section area of 14 mm × 9 mm were glued together with EAA (0.875 g EAA powder/1 mL water), and it could afford a weight of up to 1.5 kg (applied adhesive strength is 116.8 kPa, Figure 3B; Movie S3, Supporting Information). Sealed with Vaseline, the glue also showed exceptional wet adhesion strength. The EAA glued glass substrates were immersed in water up to 3 d, and a thin layer of Vaseline was used to cover all edges of two glass plates. After 2 d, the adhesion area was still able to afford a force of 6 kg (Figure 3C; Movie S4, Supporting Information). After 3 d of immersion in water, the affordable force of the glued glass plates slightly dropped to 5 kg, maintaining up to 83% of its adhesion capacity under water.

The mechanism of egg white albumen adhesive was interpreted in Scheme 2. From the view of primary structures, native egg white albumen generally is comprised of ≈90% water and ≈10% proteins, including ovalbumin (54%), conalbumin (12%), ovomucoid (11%), globulins (8.0%), etc.[30] The covalent bonding or chemical reactions did not play an important role in the interface interactions between egg white albumen proteins and substrates. (The disulfide bonds in egg white would not react in current circumstance without free thiol groups or reducing agents.) Due to irreversible protein aggregation formed in the air-drying process, the adhesion mechanism could be explained by hydrogen bonding network formation and conformation changes of egg white albumen proteins. In a pristine status, egg white albumen proteins were well stabilized and dispersed in solution in the major secondary structures of α-helix and unordered coil (α-helix (40.6%), unordered coil (28.2%), β-sheet (15.8%), and β-turn

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Figure 1. Egg albumen adhesive: A) EAA (0.875 g EAA per mL H2O); B) EAA pinned between two fingers (0.875 g mL-1); C) EAA was injectable and showed adhesive-induced long consistence from the syringe needle (0.875 g mL-1); D) shear-thinning behavior of EAA; E) SEM image of EAA adhesive coated on substrates.
During the air-drying process, egg white solution shrunk and well-dispersed proteins were pushed together, where hydrogen bonding interaction between proteins increased. Finally, due to loss of water, protein chains entangled together, and the hydrogen bonding interaction between polypeptides and water was replaced by intramolecular/intermolecular hydrogen bonding interaction of polypeptide, and hydrophobic parts would aggregate together as well, leading to the formation of heavily entangled protein aggregates with irreversibly crosslinked network.

The secondary structures of dried egg white albumen were characterized by analyzing the amide I region peak of Fourier transform infrared spectroscopy (FTIR) spectra. Raw spectra were self-deconvoluted to overlapped single peaks, and then Gaussian fitting was conducted for all peaks and the secondary structure fraction could be calculated based on the fitted peaks' area (All peaks must comply with the corresponding secondary derivative trace). Compared with native solution status, the fractions of $\alpha$-helix and unordered conformation dropped to $14.8\%$ and $17.0\%$ respectively, whereas the $\beta$-sheet and $\beta$-turn...
jumped to 48.2% and 20.0% dramatically (Table S1, Supporting Information), indicating high degree of inter-/intramolecular hydrogen bonding interaction between different protein backbones that lead to highly crosslinked hydrogen bonding network. When the grinded dry egg albumen powder was mixed with water, the polypeptide crosslink network would be swollen rather than dissolved despite partial replacement of intramolecular/intermolecular hydrogen bonds of peptide chains by water molecules, which could not break the entangled protein network driven by hydrogen bonds and hydrophobic microdomains.[33] During the swelling, the polypeptide chains on different grinded grains got high mobility to interpenetrate each other to form gel-like adhesive, which could build strong interactions with substrates by hydrogen bonds and van der waals force under applied pressure.[34] From the FTIR spectra in Figure 4A, with existence of water, amide band II, III, and N–H stretching peak shifted to left obviously, and the weak peak at 3070 cm$^{-1}$ disappeared due to deprotonation of amine moieties with water, indicating the hydrogen bond formation between egg white albumen and water molecules, which partially broke the protein chain hydrogen bonds network. Based on the secondary structure analysis of EAA glue (0.875 g dry powder per mL water) in Figure 4C, the fraction of $\beta$-sheet conformation did not drop to the level of the native solution, but accounted for 55.0% of total secondary structures (15.5% $\alpha$-helix, 14.2% unordered, and 15.3% $\beta$-turn, see Table S2 in the Supporting Information). It suggested that albumen protein backbones in EAA glue still kept aggregated morphology rather than native well-stabilized solution status. The secondary structure analysis result is consistent with the result the egg

Scheme 2. The schematic mechanism interpretation of pressure sensitive egg white albumen adhesive (EAA).

Figure 4. A) FTIR-ATR spectra of EAA powder and EAA-water adhesive, b) secondary structure analysis of dry EAA from amide I region of FTIR spectra, c) secondary structure analysis of EAA–water adhesive from amide I region of FTIR spectra.
white albumen recombination experiment, indicating the irreversibly crosslinked network induced by hydrogen bonding interaction. The hydrogen bonds between polypeptide chains in egg white albumen adhesive could be further confirmed by employing urea to cleave those intermolecular/intramolecular hydrogen bonds.[35] Compared with same concentration egg employing urea to cleave those intermolecular/intramolecular hydrogen bonds.

To evaluate EAA's wound closure performance in vivo, a rat model was employed to investigate the healing effects of EAA coated PCL nanofibrous patches on skin tissue wound sites. PCL nanofibrous membrane coated with EAA was adhered to rat's skin to cover the whole incision area for wound site repairing. Only 5 min later, it was observed that the corresponding wound site was well glued and thereafter left for healing investigation (Figure S5A–D; Movie S5, Supporting Information), which was compared with wound sites endured with regular suture or hemostasis only treatments for recovery.

After 5 d, all the three wound sites were examined to gain an insight on the healing process and to monitor the changes in wound closure (Figure 5E). Both wounds treated with EAA adhesive (a) or medical suture (b) were found to get good recovery, compared with the control group processed by hemostasis treatment only. The wound sites endured different closure treatments recovered well after 5 d, and showed excellent healing recovery effect. Around EAA treated wound sites, there was not any suspicious sign of infection or inflammation observed, while all samples of the blank group without closure treatment showed dehiscence (Figure 5E). Therefore, it can be concluded that the facile EAA glue treatment exhibited comparable healing performance to conventional suture closures on skin tissue wound sites, and EAA did not lead to any obvious wound infection or inflammation.

Histological studies were conducted to assess in vivo wound healing effects and possible side effects on the rats' skin tissues for EAA treatment. The incisions treated with EAA showed longitudinal collagenous fibers, sporadic neutrophils, and fibroblasts beneath the interfaces. Epithelium consecutively integrated with basement membrane and there were no deep openings left in the tissues. Moreover, hair regrowing was observed across the incision without scarring, suggesting the overall healing of wounds promoted by EAA without any obvious side effects (Figure 6A,D). In sutured wound sites, less anomalous collagen fibers, more neutrophils and fibroblasts (Figure 6B,E), and less hair regrowing were found, compared with similar wounds treated by EAA. The incisions only treated with hemostasis were found to be filled up with large amounts of granulation tissues, and certain mass of polymorph nuclear leukocytes, macrophages, fibroblasts and blood capillaries was found in the tissues (Figure 6C,F). Therefore, the incisions treated with EAA got better recovery than that treated with conventional sutures, suggesting the good perspective of EAA glue in wound closure.

EAA was slowly degraded and still visible after 35 d of implantation. At 7 d after implantation, a moderate acute inflammatory response was observed in the outmost layer with the presence of representative multinuclear cells; and a granulation tissue with fibroblast proliferation and few loose collagen layer formation were showed around the albumen (Figure 7A,D,G). After 21 d, EAA began to lose structural integrity and almost was filled by invading cells. The inflammatory response decreased with the disappearing of the leukocytes and increasing of macrophages (Figure 7B,E), and a thin fibrous capsule isolated the albumen from the local tissue by the foreign body reaction, which included a few macrophage eroding the albumen surface and a collagen encapsulation consisting multilayers fibroblasts (Figure 7H). The host response against the EAA became minimal with increasing implantation time. Moreover, vascularization was observed in the periphery fibrous tissue. At 35 d after implantation, more mature vessels could be observed around encapsulated smaller pieces of degraded EAA (Figure 7C,F), a thicker fibrous capsule was formed around the whole implant and surrounded collagenous connective tissue (Figure 7I). From previous report, egg induced allergy affects 1%–2% children generally.[36] Although our in vivo skin adhesion and subcutaneous implantation in mice did not show significant immune-response, it should be cautious while the EAA is applied to human body. A further research should be conducted for its potential immunogenicity.
3. Conclusion

EAA glue from fresh eggs can be fabricated via the simple processes of air-drying, grinding and mixing with proper amount of water. The obtained EAA exhibited outstanding shear adhesion performance among current popular commercial medical adhesives on various types of substrates, as well as good underwater adhesion performance. EAA also displayed excellent wound healing performance in vivo experiments on rats, and did not show strong long-term inflammatory response in vivo subcutaneous implantation degradation experiment. Considering its abundant source, simple and environmental friendly fabrication process, inherent nontoxicity and biocompatibility, and extra low cost, EAA could be a...

Figure 6. Histopathological evaluation: A,D) treated with EAA; B,E) treated with suture; C,F) hemostasis only for blind control group (100×).

Figure 7. Degradation of subcutaneously implanted egg albumen in rats. Egg albumen was harvested at 7, 21, 35 d postoperation and stained with A–F) H&E and G–I) Masson trichrome stain, respectively. Bars in A–C,G–I) 200 μm and D–F) 50 μm.
medical adhesive candidate with brilliant perspective in clinic medication.

4. SEM Characterization

SEM characterization was performed by using a JEOL-5900 scanning electron microscope. A thin layer of gold (thickness <10 nm) was coated on samples by sputter coating.

4.1. Lapse Shear Adhesion Measurement

A universal tensile tester (Instron 5965) was employed to determine the lapse adhesion of EAA and compared with different types of commercial adhesives on different substrates, including glass, PDMS and pigskin tissue. PDMS substrate was prepared by curing of SYLGARD184 silicone elastomer at 80 °C. Pigskin tissue substrates were freshly prepared by thawing the stored bulk product at room temperature for 1 h, and hairs and fat parts were removed from the tissue before test. All substrates were cut to a rectangular shape of 75 mm × 25 mm and the overlapped area with adhesives was 25 mm × 20 mm. EAA powder and water were mixed homogeneously with using of a spoon at the ratio of 0.875 g EAA /1 mL water. Prepared EAA adhesive of ~200 µL was casted onto a piece of substrate and covered with another piece of substrate under moderate pressure. Similar amount of commercial adhesives were casted on control groups for comparison. The curing time for all samples was 5 min at room temperature under moderate pressure. The shear adhesion was determined using of Instron tensile tester at a strain rate of 10 mm min⁻¹, and 5000 N load cell was used for glass substrate (500 N for pigskin/PDMS substrates). Each test was repeated for three times.

4.2. Rheology Test

Rheology frequency sweeping test was performed on a TA DISCOVERY HR1 hybrid rheometer, with a cone plate (20 mm diameter, 2° angle, steel). The strain was set up as 0.2%, and frequency increased from 0.1 to 100 rad s⁻¹ by steps.

4.3. FTIR Characterization

FTIR-attenuated total reflection (ATR) was carried out on a Nicolet iS10 FTIR Spectrometer, and 64 scan per sample with a resolution of 4. For the secondary structure analysis, the raw spectra at the region of 1610–1700 cm⁻¹ were deconvoluted (bandwidth = 60 and enhancement = 3.0 for all spectra), and the separated peaks were fitted by Gaussian function. All fitted peaks must comply with the corresponding secondary derivative trace (smoothed by Savitzky–Golay algorithm with 11 points window).

4.4. In Vivo Wound Healing Test

All the animals used in this study were purchased from Nanjing Medical University Experimental Animal Center. All animal procedures were approved by Animal Care and Use Committee of Nanjing Medical University. Six male SD rats (200–250 g) were anesthetized with chloral hydrate (300mg kg⁻¹). Three incisions (2 cm) were cut on the back of animals after sterilization, and treated respectively using one of following methods—suture closure, adhesion closure with EAA coated PCL patch, or hemostasis only (control group). 4-0 unresorbable suture was used to close the wound. The wound incision was covered with a piece of PCL electroposn membrane (2 cm × 1 cm) coated with ~100 µL EAA. All egg albumen powder and PCL nanofibrous membranes were sterilized under UV exposure for 3 h before applying on animals.

After 5 d postoperation, PCL patches were slowly torn off, and each rat was sacrificed and three rectangle pieces of skin tissues (2 cm × 1 cm) containing the wound were resected. Samples were then fixed in 10% neutral buffered formalin solution for 24 h. The section of middle parts of scar was stained with hematoxylin–eosin (H&E) and evaluated using an optical microscope.

4.5. In Vivo Degradation Test

In vivo animal experiments were performed in rats weighting 350 ± 50 g. All experiments were conducted under National Institutes of Health protocols and were approved by the Ethics Committee of Southern Medical University. Under deep inhalant isoflurane general anesthesia, the rats were put in prone position and dorsum was aseptically prepared for surgery. 1.2 cm long skin incisions at abaxial to the vertebral column were created, and the underlying subcutaneous tissue was separated to provide sufficient pocket room for EAA, following the implantation, the skin was closed with absorbable suture. At selected time postoperation (days 7, 21, 35), the gel with surrounding tissue and whole skin were harvested, fixed in 4% paraformaldehyde solution for 72 h, dehydrated by graded alcohols, and embedded in paraffin. Section of 5 μm was used for H&E and Masson trichrome stain.

5. Experimental Section

Materials: All eggs used in the research were purchased from local food marts. PCL was purchased from Sigma Aldrich (MW = 70 K). Pigskin tissue and rib bones were ordered from local farm market and stored at −20 °C before use. Commercial cyanoacylate medical adhesive glue was purchased from Guangzhou Baiyun Medical Adhesive CO., LTD. Commercial fibrin glue was purchased from Hangzhou Puji Medical Technology Development Co., Ltd. Sylgard 184 PDMS was purchased from Dow Corning, and PDMS sheets were prepared according to the curing protocol stated by the supplier.

Methods—Preparation of EAA: Briefly, egg albumen was taken from fresh eggs and transfered to a petri dish using a pipette for overnight air-drying. The albumen was then grinded into fine powder in a mortar and stored at room temperature for further use. For example, 4.58 g fresh egg albumen was transfered to a petri dish (35 mm diameter) and air-dried in fume hood overnight (airflow rate: 100–110 fpm). The air-dried egg white looked like light yellow brittle bulky solid, and could easily be broken to pieces and then grinded into powder in a mortar in ambient environment. After in vacuum oven at room temperature, the dry sample was weighted. By comparing with the weight before drying, the water content of the powder was calculated. The sample was
measured every 24 h until the weight loss is less than 0.1%. Based on the measurement, the overnight air-dried egg white has 5.0% residual water left. EAA glue was freshly prepared by mixing certain amount of the powder and double deionized water (double deionized water was directly taken from Direct-Q 3) before use. For example, 70 mg powder and 80 µL H₂O were mixed uniformly using a spoon to obtain viscous EAA glue at the concentration of 0.875 g EAA per mL H₂O.

Methods—Preparation of PCL Nanofibrous Membrane: Electrospinning nanofibers were prepared as per the protocols described in our previous reports.[37] Briefly, 500 mg of PCL was dissolved in 5 mL mixture of DMF and DCM (1:4) at a concentration of 10%, and added into a syringe mounted on a syringe pump (PH2000 Infusion). The positive lead from a high voltage (20 kV) supply (GAMMA, High Voltage Research) was connected to ground. The distance between the needle and the stainless steel-mesh was used to collect PCL nanofibers. The steel-mesh was mounted on a syringe pump (PH2000 Infusion). The positive lead from a high voltage (20 kV) supply (GAMMA, High Voltage Research) was connected to the needle via an alligator clip. A piece of 15 cm × 15 cm stainless steel-mesh was used to collect PCL nanofibers. The steel-mesh was connected to ground. The distance between the needle and the mesh was 15 cm. The rate of infusion for PCL solution through syringe was set to be at 1 mL h⁻¹ to get a thickness of 50 µm for future use.

Methods—Application of EAA and PCL Nanofibrous Membrane: In order to improve operability of albumen glue for applications, an electrospinning PCL nanofibrous membrane was used as a substrate to hold the glue. Briefly, EAA was uniformly coated on the surface of PCL membrane (~100 µL on an area of 2 cm × 1 cm) with using a spoon.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest
The authors declare no conflict of interest.

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