Egg Albumen as a Fast and Strong Medical Adhesive Glue

By

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Abstract

Current sutures can cause inflammatory responses, which prolong healing time and increase operation complexity, even worse induce stress concentration on fragile tissues. Medical adhesives treatment is a promising way to close tissue via a minimum invasion in surgical operation, and operated simply by surgeons. In this project, an egg albumen adhesive (EAA) with ultra-high adhesive strength, fabricated from fresh egg via straightforward process of air-drying, grinding and mixing with water, has been easily used to bond various types of materials without any chemical and physical modifications. Here I demonstrate that EAA exhibited stronger adhesive property on different substrates, including pigskin tissue, glass, and polydimethylsiloxane by compared with popular commercial medical adhesive, cyanoacrylate synthetic glue and fibrin glue. The egg albumen adhesive also showed exceptional underwater adhesive strength. Finally, EAA displayed excellent wound healing performance and did not show strong long-term inflammatory response in vivo experiments on rats, suggesting its potential as a medical glue, considering its abundant source, simple fabrication process, inherent non-toxicity, and low cost.
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<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
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<tr>
<td>DDW</td>
<td>Double Deionized Water</td>
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<tr>
<td>EAA</td>
<td>Egg Albumen Adhesive</td>
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<tr>
<td>FT-IR</td>
<td>Fourier transform infrared</td>
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<tr>
<td>ATR</td>
<td>Attenuated Total Reflectance</td>
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<tr>
<td>HE</td>
<td>Histopathological-eosin</td>
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<td>PCL</td>
<td>Polycaprolactone</td>
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<td>PDMS</td>
<td>Polydimethylsiloxane</td>
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<td>SEM</td>
<td>Scanning Electron Microscope</td>
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Chapter 1 Introduction

1.1 Background

Surgical sutures have been played a critical role in the surgical operations since the breakthrough surgery in medical sciences. Traditional surgical sutures are not reliable because old-way sutures cause surgical site infection. For example, the postoperative infections are increased to 4.15% higher after artificial joint replacement due to the unreliable antibacterial ability of suture materials [1]. In addition, surgical site infections would increase clinical burden and healthcare cost for patients, because the infections could result in prolonged wound healing and wound dehiscence [2]. In order to perform nearly perfect quality of sutures, many researches have been developed and focused on improving the latest sutures’ techniques so far [3, 4]. Recently, the main advancements have been achieved in minimising the side effectiveness and the cost of the sutures by evolving the sutures procedures and materials, such as bacterial-adhesive sutures [5-7], antibacterial-coated sutures [8-10], and absorbable polymeric and bidirectional barded sutures [11-13]. However, these sutures still may cause inflammatory responses, scar tissues, and injured surrounding tissues sometimes; as well, the complex suture procedures increase operation time.

Wound closure techniques based on biomedical adhesives aim to accelerate wound healing, decrease operation time, and alleviate patients’ pain. The biomedical adhesive can close wound with shorter operation in a facile procedure and avoid further injure the wounds result by using a minimum invasive method. Adhesive materials are the
most important part that researchers are focusing on recently. The development of biomedical adhesive materials has experienced from synthetic to staples and biodegradable ones. These bioadhesives can be classified based on material types into followings: synthetic polymers- polycyanoacrylate medical adhesives [14, 15], polysaccharide-chitosan bioadhesives [16-18], polyurethane based adhesives [19, 20], polyethylene glycol medical glues [21, 22], polyesters adhesives [23, 24]; natural protein derived-fibrin biomedical adhesives [25-27], albumin-derived adhesives [28-30], gelatin based adhesives [31, 32], collagen bioadhesives [33, 34], hyaluronic acid adhesives [35, 36], dextran tissue adhesives [37, 38]; and biomimetic mussel-inspired dopamine bioadhesives [39-42], etc.

The ultimate’s goal for medical bioadhesives is to have a strong adhesive strength, low cost, biocompatibility, and simple operation procedure. The current adhesives either exhibit lower adhesive strength (fibrin biomedical adhesives, gelatin based adhesives, collagen bioadhesives) and needs extra crosslinkers to reach the closure (glutaraldehyde, NHS [43], thiol [44], photo crosslinker [45]), or with strength but noticeable toxicity in fast polymerization (cyanoacrylate [46]). Inspired by mussel’s firm adhesion to rocks under the tough ocean environments, dopamine based adhesive glues have been developed and are gaining enormous attention [39, 47, 48]. Though dopamine-based adhesives show good strength, there is a major concern while using it, the chemical oxidants are needed to activate dopamine, and thus cause potential cytotoxicity [49-51].
Egg albumen is one of the most favorable food in the nature because of its biocompatibility, biodegradability, and non-toxic nature to human body [52]. In addition, the ease of availability from eggs and cost friendly prices are a great step towards conserving environment by using renewable resources. Furthermore, egg albumen has not been investigated yet for medical adhesive applications.
1.2 Problem Definition

Surgical suture has been researched and developed for decades of years. Since now, many kinds of advanced sutures’ materials, such as biodegradable materials, bioabsorbable materials, and antibacterial materials, have been made and ultimate. As well, the suture techniques have been improved recently. However, the surgical sutures are still not perfect. Sutures in most forms usually lead to inflammatory responses, prolong healing time, left scar tissues, and injured surrounding tissues. These drawbacks cost much money for further healthcare, and cause extra pains for patients. In order to change this situation, sutureless techniques (for example wound closure technology) are being developed.

Wound closure, which is based on medical adhesives, is known as the most important sutureless technique, it can decrease operation time, accelerate healing, and reduce the wound tissue stress to avoid further injure the wounds tissues. However, recent medical adhesive materials either have low adhesive strength, or are toxicity to human body. In addition, despite some medical adhesives have great adhesive strength, these adhesives cause unexpected issues, such as can be hardly removable from surgical instruments, adhere wound tissues too strongly then cause wound further damage, or glue surgical dressing on wound accidently. Otherwise, no adhesive materials are made from real raw natural materials directly.

Otherwise, egg albumen as natural material, has been used for controlled release biomaterials, and synthesizing bio-film and hydrogel, but no one has researched it as
medical adhesives so far [52-54].

In this project, combine issues as described above, and inspired from the raw egg albumen’s high viscosity, a low-cost egg albumen adhesive with unique properties—exceptional adhesive strength, maintaining underwater adhesive, biocompatibility, and bioresorbable has been synthesised. Furthermore, egg albumen adhesive show great adhesive behavior on animal wound healing test in vivo; it exhibits excellent adhesive ability in water condition.
1.3 Objectives

The objective of this project is using a simple and economical way to design a biocompatible and biodegradable medical adhesive for wound closure. Furthermore, it aims at studying on obtaining higher adhesive ability than current popular commercial medical glue, and even keeping good adhesive behavior in underwater environment. Also, the applicable results in vivo can be obtained by combining with nanofibers mesh. To reach these objectives, the work is established as:

1. Fresh egg albumen was taken for air-dried, then grinded in mortar for egg albumen powder.
2. Mix with quantitative double deionized water to obtain strong adhesive ability.
3. Adhere glass slides, fractured bones, pigskins and lift heavy objects to test its adhesive ability.
4. Underwater adhesive test to prove its reliable adhesive ability in moist environment.
5. Tests in vivo to evaluate its wound closure ability, biocompatibility, and biodegradability.
1.4 Summary of Experimental Methods and Major Findings

1.4.1 Summary of experimental methods

In this project, the fresh egg albumen was air dried for 12 hours and grinded as powder, and then, mixed with double deionized water. Furthermore, the mixed egg albumen adhesive was used glue the pigskin and cut wounds on rats for wound closure application. Then, the chemical properties were examined by Fourier transform infrared (FTIR-ATR) spectroscopy and UV/Visible Spectrophotometer (Ultrospec 4300 pro) techniques. The surface topography was characterized by optical microscope (Motic-BA310) and scanning electron microscope (SEM). The shear strength of egg albumen adhesive was tested by universal tensile tester (INSTRON 5965). In addition, the adhesive behavior was evaluated by using rheology frequency sweeping test (TA DISCOVERY HR1 hybrid rheometer). At last, the wound closure ability, biocompatibility, and biodegradability were checked by in vivo test on rats models and hematoxylin and eosin Staining (H&E) analysis.

1.4.2 Summary of major findings

Raw egg albumen exhibited ultra-high adhesive strength and showed great injectability from a syringe needle with great formability after air dried, grinded, and mixed with double deionized water. Egg albumen adhesive presented stronger adhesive strength than other popular commercial adhesives, especially, the adhesive ability is extremely excellent on glass
substrates. As well, the adhesive strength of egg albumen adhesive is reliable on pigskin tissue and rats’ skin wounds.

Egg albumen adhesive can still keep its great adhesive ability in underwater condition on glass substrates even after 3 days.

Egg albumen adhesive showed good biocompatibility and biodegradability in vivo.
1.5 Thesis Layout

This paper contains six chapters. The organization is shown as follows,

✧ Chapter 1 expresses the brief introduction of background, problem definition, objectives, methodology, and major findings,
✧ Chapter 2 shows a general literature review on surgical sutures, medical adhesives, egg albumen science,
✧ Chapter 3 presents detailed experimental procedures and used instruments,
✧ Chapter 4 provides the results and discussion,
✧ Chapter 5 concludes the major findings and the summary of the project,
✧ Chapter 6 exhibits suggestions for the future works.
Chapter 2 Literature Review

2.1 Surgical Sutures

Surgical suture, as one essential part of traditional surgical science, as well as one of the most critical wound closure techniques, has been developed for decades of years. Surgical suture is defined as ligating blood vessels and related tissues together by using biomaterials (natural or synthetic materials) in order to closure wounds and stanch bleeding [55]; then, the major function of the suture is worked as sewing separated tissues. The goal of that is to help wounds closure as fast as possible in the way of minimal side effect and cost on patients. While researchers never stop searching ideal suture, which should be biologically inert to humans totally and should not lead any side effects; in another words, the ideal suture should own all characteristics as following: easy to operate by surgeons and sterilize before using, minimum tissue reaction, anti-bacterial, contain high tensile strength, no allergically response, no toxicity for human body, absorbable or degradable after utilizing their functions (can dissolve in human body fluids after using) [56-59]. To approach this ultimate goal, many advanced suture techniques, include absorbable polymeric sutures, bidirectional sutures, antibacterial-coated sutures, and bacterial-adhesive sutures, have been researched so far.
2.1.1 Recent advanced suture techniques

2.1.1.1 Absorbable polymeric sutures

Generally, the absorbable polymeric suture is named as suture technique based on absorbable polymeric materials. Since the first absorbable material (polyvinyl alcohol) for suture using was synthesized in 1931 [60], many advanced types of absorbable materials have been came out in recent 80 years. Those materials mainly include polyglycolide, poly(glycolide-L-lactide), polyglyconate, polyglycolic acid (PGA), poly(trimethylene carbonate) (PTMC), poly(dioxanone) (PDS), and etc. [61-64].

While most advanced absorbable suture materials are synthetics, this is because synthetics have better mechanical and physical performances, for example, relative higher tensile breaking strength and more elasticity than natural materials, and which both factors must be required in suture techniques [65]. Among them, PGA, as the most popular absorbable suture material, was first introduced in 1970s [66]; its great mechanical and physical properties were evaluated both in laboratory and clinical conditions. The tensile strength of PGA is great as synthetic nonabsorbable suture material for example Dacron, and even higher than nylon’s; and the rate of absorption is good as chromic catgut, otherwise, the ability of resistance to infection and inflammatory is excellent [67-71]. Due to these advantages, PGA is still using as suture material recently but with modified, for example, it shows much better tensile strength and absorbable rate if combined using PGA with poly(trimethylene carbonate), as well, the biocompatibility is outstanding [72].
2.1.1.2 Bidirectional barded sutures

Bidirectional barded suture is one kind of advanced suture technique that known as the barbs would change directions in the middle core of suture lines, as well as the suture needles are attached on both end of the suture [73]; in another word, the barbs on a same suture face in an opposite direction on both sides of middle point. To be different from others sutures methods, bidirectional barbed suture is more advanced in suture technique itself instead of using more advanced functional materials. Most conventional sutures are required of tying a knot to secure the closure; however, many complications would be motivated such as infection, tissue ischemia, or undesirable scarring, since knots may be not tight enough then break, slip, or extrude. Also, it takes time to tie knots, and training is required for surgeons. The most technique problem of traditional sutures is that the wound tension could not distribute uniformly (excessive tension or small tension) throughout the wound closure, which would cause wound dehiscence, furthermore, excessive tension would have an opportunity to result in focal ischemia and necrosis. [74, 75]

As a novel suture technique, bidirectional suture technology does not require the tying of knots, this advantage prevents all the possible complications that caused by tying knots. In consequence, due to minimally invasive advantages, bidirectional barbed suture was first applied in surgical plastic area including brow, midface, and neck surgical. [76, 77] In addition, bidirectional barbed suture is simply operated by surgeons which save time for training and during surgical.
Otherwise, both absorbable and nonabsorbable materials are suitable in using in bidirectional barbed sutures: polydioxanone (PDS) and the polyglycolide-poly-e-caprolactone copolymer (PGEC) are common used absorbable materials, polypropylene (PPy) and nylon are nonabsorbable materials.

2.1.1.3 Antibacterial-coated sutures

Recently, surgical site infections caused by bacteria are still the most challenging issue in almost all wound sutures techniques, it accounts for the most infections in surgical patients. [10] In order to solve and prevent the risk of surgical infections, the antibacterial sutures have been developed, for example, polyglactin 910 coated with Triclosan. [78] So far, the widely used triclosan, which is known as a broad-spectrum antibacterial material, has been applied on medical fields for many decades years. [79] As well, another popular antibacterial activity, Vicryl plus can vastly reduce the risk of infections and enhance the antibacterial ability when Vicryl plus is being used with triclosan-coated polyglactin 910 suture materials. [80]

2.2 Medical Adhesives

However, it has not found any suture materials or techniques that could meet all these ideal criteria so far. In order to solve problems faced in sutures and develop a relative ideal wound closure method, recently, the sutureless wound closure technology has attracted huge interest and has been researched so far. As the most popular sutureless technique, adhesive glue could provide a facile way to close wound including sealing
tissue leaks, preventing bleeding, and binding tissues within less operation time via a minimum invasion, which could avoid further wound injury and lead to a better appearance after recovery. [81-83]

Currently, common reported wound closure adhesives are classified into the following aspects: the natural protein derived: fibrin adhesives [25], albumin glue [84], gelatin [85], collagen [33, 34]; polysaccharide-chitosan [86], alginate [87, 88], hyaluronic acid [89], dextran [90]; synthetic polymers: polycyanoacrylate [91], polyurethane [92], polyethylene glycol [93]; and biomimetic mussel-inspired dopamine [39, 47, 94] etc.

The ultimate goals for ideal wound closure bioadhesives are having 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 (like glutaraldehyde [84], NHS [95], thiol [96], photo crosslinker [97]), or have good adhesion strength at the cost of noticeable toxicity (e.g. cyanoacrylate [98]).

As most popular commercial synthetic adhesives, cyanoacrylate and its derivatives have excellent sealing performance owing to its moisture-initiated fast polymerization [99-102]; however, their toxicity and potential toxicity of their degradation products are major issues in their applications [103]. Although fibrin based medical glues have great biocompatibility, their applications were limited since their adhesive strengths are not reliable.[104] Inspired by mussel’s strong adhesion to rocks in complex underwater ocean environments, dopamine based glues were discovered and gained
enormous attentions.[39, 47, 48, 105-110]

2.2.1 Cyanoacrylate medical adhesives

Cyanoacrylates were first researched in 1949 and their potential as wound closure adhesives was then fast developed. [111] Due to excellent adhesive ability, cyanoacrylate and its derivatives have been researched and gained many medical and commercial applications as tissue wound sealants and orthopedic bone cements to replace traditional sutures. In addition, there are more advantages of cyanoacrylates, includes easy and rapid to be used, relative painless, and external application offer a minimal scar tissue. Usually, as medical adhesives, cyanoacrylates’ homologues mainly include methyl-cyanoacrylate (MCA), ethyl-cyanoacrylate (ECA), isobutyl-cyanoacrylate (ICA), octyl-cyanoacrylate (OCA), butyl-2-cyanoacrylate, N-butyl-2-cyanoacrylate, and 2-octyl-cyanoacrylate (2-OCA). [112]

Despite their excellent adhesive ability and widely application, the clinical toxicity of cyanoacrylate and its derivatives could not be ignored as medical application, as well, their clinical toxicity has not been widely and fully researched. For instance, some reports proved that cyanoacrylates could induce inflammatory response and even necrosis of tissue, which may cause thrombotic because of cyanoacrylate-associated necrosis. [113-115] Besides, the toxicity of cyanoacrylate adhesive would cause postoperative arterial occlusive lesions [116, 117] Furthermore, recent animal tests showed that cyanoacrylate adhesive would cause occlusion of the intracranial arteries because of tissue inflammation. [118] Aside from clinical toxicity, many studies
showed that the cytotoxicity of cyanoacrylate and its derivatives. For example, the polymerization of cyanoacrylate is heat release process, while these exothermal has a chance to cause cell damaged in cell growth; this situation cannot be ignored even the surgical cyanoacrylate is diluted in 10 times. [119, 120] This issue still cannot be fully solved even researchers try to prevent cells directly contacting with the adhesive, and this special polymerization process is still cytotoxic to human oral fibroblast cell cultures. [121]

2.2.2 Medical fibrin glue

As another important sutureless tissue adhesive glue, the medical commercial fibrin glue contains two functional components: one is fibrinogen, and the other is thrombin. During the seal process, thrombin can convert the fibrinogen into solventless fibrins under the existence of Ca^{2+} and factor XIII. The reaction procedure is fast that makes fibrin as fast adhesive sealant. In fact, the first fibrin hemostatic factor was synthesized in the year of 1909 by Bergel. [122] After that, fibrin sealant has been fast developed and applied in many tissue fields, including ophthalmology, hernia repair, noncardiac thoracic, seroma prevention, and fistula repair. [123-127] To be different with cyanoacrylate medical adhesives, fibrin glue gains no toxicity and cytotoxicity since it is partially human-derived product; also, fibrin adhesive glue is permitted by the FDA as commercial safe adhesive that is using to closure tissue injured as its nonharmful nature. [128]

Fibrin glue would be the ideal wound closure agent, but the adhesive ability is relative
low compared to cyanoacrylate adhesives and other medical adhesives. For example, researchers have created the animal models by applying fibrin adhesive glue for implanted cartilage sealed on knee joints, but the results showed that fibrin adhesive was too weak to bear the joint loading, and it finally failed because of shear damage at the cartilage interface. [129]

### 2.2.3 Dopamine based medical glue

As an important and widely used biomaterial, dopamine has been drawn a lot of attentions since it was first discovered. Dopamine, as one of the human neurotransmitter, is main paste component of mussel foot protein; the research showed that protein contains 3,4-dihydroxy-L-phenylalanine (dopamine) exhibit adhesive ability. As a mussel-inspired biomaterial, dopamine is widely used in bioresearch. Poly-dopamine, as dopamine derived synthetic polymer, can be formed by dopamine self-polymerizing under alkaline conditions, since the catechol functional group contains on dopamine could be oxidized to quinone under alkaline conditions (Figure 1), which makes it to be polymerized and create a very thin layer upon the surface of object, while this dopamine layer exhibits adhesive due to covalent bond, hydrogen-bonding, metal chelation, and π-π interactions, and also because poly dopamine contains a lot of amine and hydroxyl groups. [130, 131]
Figure 1. Dopamine self-polymerized to polydopamine in alkaline environment.
The self-polymerization’s mechanism of dopamine is that the catechol groups can be oxidised in the alkaline basic environment. [132] Poly-dopamine is widely used in many kinds of applications, like the modifications of surface, bio-inspired hydrogel, metal depositions, and delivering the drugs [133-137] because of its great physicochemical possessions and high biocompatibility [138, 139], and the most popular property is its mussel-inspired adhesive ability. For example, the mussel-inspired polydopamine coated on a gelatin/ PCL nanofiber membrane was used for gastric wound healing. [140] Dopamine is also widespread used to modify and insert man-made functional groups into some polymers’ side chains for gaining simulated adhesive biomaterials; for example, dopamine was reacted on FDA-approved poly(ethylene glycol) for achieving adhesions ability. [141]

2.3 Egg Albumen Science

Eggs, as one of the most popular daily food in nature, are used throughout the whole world. Eggs and egg-related products have been played an important role as diet protein intake for human beings since eggs’ high nutritional value. Egg albumen hold around 58% of total egg’s weight and contributes about half of the total egg protein [142]. Egg albumen contains of almost 90% water, about 10% proteins, 0.5% carbohydrate, and the rest of other things [143], and this majority of water content makes albumen fluid like. Proteins in egg albumen include ovalbumin, ovotransferrin, ovomucin, lysozyme, and ovoglobulins, and these egg albumen proteins show great functional properties, such as gelation, foaming, and emulsification [144, 145]. Since
proteins are the most solutes and very little other particulate ingredients contained in egg albumen, which make egg albumen relatively homogeneous, then these excellent properties result in egg albumen available for biological studies [146]. In addition, the friendly cost, good biocompatibility, biodegradability, and non-toxic nature to human body are a great step towards leading egg albumen as great biomaterials.

2.3.1 Proteins ingredients of egg albumen

Ovalbumin, constituting 54% of total proteins, is the most plentiful protein in egg albumen. Ovalbumin exists as glycoprotein monomer, and the molecular mass weight is 44.5 kDa, also the isoelectric focusing point is 4.5 [147]. As well, ovalbumin is an egg albumen protein only that filled with free sulfhydryl function groups [148]. Three polymorphic ovalbumin forms are known so far, which are the ovalbumin contain two phosphate groups, the ovalbumin contain one phosphate, and no phosphate groups per molecule, respectively [149, 150]. All these three ovalbumins do not show any remarkable differences of chemical properties.

Ovotransferrin, which comprises 12% of the egg albumen proteins, is also a glycoprotein, and its molecular weight if 77.7kDa and has an isoelectric point of 6.1 [151]. Usually, ovotransferring is treated as the bacteria-inhibiting and iron-binding protein in egg white, in this way, this protein is generally functioned as the iron transportation [152, 153]. In addition, the ovotransferrin contains a lot of disulphide bridges, which giving it high stability because of its six disulphide bridges in N-area and nine disulphide bridges in C-area [154].
Ovomucin contributes 1.5% only of the whole protein in egg albumen, but it acts a critical part for holding the structures of egg albumen, and its gel-like nature highly responds for egg albumen’s high viscosity; all these are because of ovomucin’s viscous sulfated glycoprotein structure [155]. Also, the ovomucin content affect the viscosity of egg albumen directly, which arise the ovomucin content can increase the viscosity. While, the ovomucin protein is difficult to dissolve in water for soluble preparation because of its high amounts of disulphide bridges [156]. There are two types of ovomucin after fractionated, one is α-ovomucin, and the other is β-ovomucin. In addition, β-ovomucin is mainly accountable for the ovomucin’s gelatinous form, which because high carbohydrate contents (58%) in β-ovomucin comprises N-acetylgalactosamine, galactose and sialic acid [155, 157, 158].

Lysozyme is a relative small sized protein in egg albumen, it has the molecular weight of 14.3kDa, also the isoelectric point is 10.7; and lysozyme contains 129 pieces of amino-acid residue, also has 4 disulfide bridges [159]. The evidence shows that lysozyme can lyse Gram-negative bacteria which is because the hydrolyzing function, and the enzyme activity of lysozyme can cleave peptidoglycans of the bacteria [160]; then it plays a critical role as protecting eggs from bacteria invading. In addition, the lysozyme has the catalysis because of the precise amino acid residues contents [161].

There are 3 types of ovoglobulins, which are G1, G2, and G3 in egg albumen. G1 ovoglobulin is treated as lysozyme among them; and G2 and G3 ovoglobulins constitute 4% protein each in egg albumen, and both ovoglobulins have molecular
weights of 49.0kDa [162]. While, researches showed globulins are not contributed to any physical or chemical properties and morphologies for the egg albumen, and it only exhibits the genetic variants of G2 and G3 globulins [163].
Chapter 3 Experiment Procedures

3.1 Introduction

This chapter describes detailed experimental procedures for the project. In Section 3.4, chemical property analysis by Fourier transform infrared (FT-IR) spectroscopy are presented.

3.2 Materials

All eggs used in this project were purchased from local food mart; egg powder was prepared from fresh egg albumen by air-drying in fume hood for 12 hours. Polycaprolactone (PCL) was purchased from Aldrich (MW=70K); PCL nanofibrous membrane was synthesised through electrospinning method. Pigskin tissue was purchased from the local farm market and stored at -20°C, and fat part was removed before using. Commercial cyanoacrylate medical adhesive glues were bought from Guangzhou Baiyun Medical Adhesive CO., LTD. Fibrin based commercial medical glue was purchased from Hangzhong Puji Medical Technology Development Co., Ltd. Sylgard 184 polydimethylsiloxane (PDMS) was purchased from Dow Corning, and PDMS sheets were prepared according to the curing protocol stated by the supplier. Urea and ethyl alcohol was purchased from VWR. Double deionized water was directly taken from Direct-Q® 3. All chemicals were used without further purification. Microscope slides were used as glass slides substrates (Purchased from Sigma). Cell culture dishes were used to prepare egg albumen powder (35mm diameter), and
3.3 Sample Preparation

3.3.1 Preparation of egg albumen adhesive (EAA)

Egg albumen was first taken from fresh eggs and transferred to a petri dish using a pipette for overnight air-drying, which was grinded to white fine powder in a mortar and stored at RT for further use. For example, 4.58g fresh egg albumen was transferred to a petri dish (35mm diameter) and air-dried in fume hood overnight (airflow rate: 100-110fpm). The air-dried egg white looked like light yellow brittle bulky solid, and can easily be broken to pieces and then grinded into white fine powder in a mortar in ambient environment. The residual water of air-dried powder was weighed by drying air-dried powder in vacuum oven at room temperature for 1 week. The sample was weighed every 24h 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 EAA powder and double deionized water every time before use. For example, 70mg powder and 80μl H₂O were mixed uniformly using a spoon to obtain viscous EAA glue at the concentration of 0.875g EAA/ml H₂O.

3.3.2 Preparation of PCL nanofibrous membrane

PCL nanofibrous membrane was prepared based on electrospinning method. At first, 500mg of solid PCL particles were melted in 5ml combination 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 wire from the 20KV voltage supply (GAMMA, High Voltage Research) was attached to metal needle via an conductive clip. Then, a piece of 15cm × 15cm 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 15cm. The rate of infusion for PCL solution through syringe was set to be at 1 ml/h to get a thickness of 50µm for future use.

3.3.3 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 EAA. The albumen glue was uniformly coated on the surface of PCL membrane (~100µl on an area of 2cm × 1cm). Thus formed egg albumen adhesive coated PCL membrane was then, placed on the cut wound site (50mg egg albumen powder/ membrane).

3.4 Characterization of Egg Albumen’s Chemical Property

3.4.1 Sample preparation

Five groups’ samples were prepared for characterization. These five groups are air-dried egg albumen powder, freeze-dried boiled-egg-albumen powder, and mixtures of 70mg egg albumen powder that was dissolved into 80µl double deionized water, 80µl urea (8M), and 80% v/v alcohol, respectively; then, all five groups of samples were freeze-dried by lyophilization again. The air-dried egg albumen powder was
prepared by taking fresh egg albumen and leaving it in fume hood overnight then grinding it as powder. For the freeze-dried boiled-egg-albumen powder, fresh egg albumen was first taken from egg, and mixed with double deionized water then heated until boiled, and grinded as powder. These five groups’ samples were all used for FTIR-ATR test. In addition, the air-dried EAA that was coated nanogold layer by gold spinning coating machine, was used for SEM characterization.

3.4.2 Chemical solvent preparation

Urea (40ml, 8M) solution was prepared by dissolving 19.218g urea into 25ml double deionized water; 70%, 75%, and 80% alcohol solution were prepared by mixing 7ml, 7.5ml, and 8ml pure ethanol with 3ml, 2.5ml, and 2ml double deionized water, respectively.

3.4.3 FTIR-ATR characterization

Fourier transform infrared-Attenuated total reflection (FTIR-ATR) spectroscopy was conducted for characterizing air-dried egg albumen powder, freeze-dried boiled-egg-albumen powder, EAA (mixture of 70mg egg albumen powder and 80µl double deionized water), and samples of egg albumen powder mixed with 8M urea and 80% v/v ethanol (70mg powder mixed with 80µl solution). The FTIR spectras were documented on the Nicolet iS10 FTIR Spectrometer, and 64 scans per sample with a resolution of 4. For the secondary structure analysis, the raw spectra at the region of 1610~1700cm\(^{-1}\) 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).

### 3.4.4 Transmittance visible spectra

The transmittance visible spectra of raw egg albumen liquid and 10% recombinant egg albumen solution was checked and compared by using Ultrospec 4300 pro UV/Visible Spectrophotometer. Raw egg albumen liquid was taken from fresh egg white directly, and the recombinant egg white albumen solution was prepared by mixing air-dried egg white albumen powder with double deionized water at the concentration of 10% (which is similar to raw egg white albumen solution), which was dispersed in ultrasonic bath at ambient condition for 4h.

### 3.4.5 Surface topography

#### 3.4.5.1 Optical microscope

The surface topography of EAA was firstly observed by using Motic-BA310 optical microscope. The optical microscope was used to evaluate the topography appearance of EAA and compared to natural fresh egg albumen.

#### 3.4.5.2 Scanning electron microscopy (SEM)

The exterior surface topography of egg albumen adhesive was further examined via a JEOL-5900 scanning electron microscope. The EAA was first prepared, and then put
in -20 °C freeze for 6 hours then freeze-dried by lyophilization. The freeze-dried sample was spin coating with a layer of gold (thickness < 10nm) via sputter coating before examination. The SEM was then applied for observing the further topography of EAA adhesive coated on PCL nanofibrous membrane.

3.5 Adhesive Behavior Evaluation

3.5.1 Sample preparation

First of all, 0.350g air-dried egg albumen powder was dissolved into 0.4ml double deionized water, and stirred by a spoon until the mixture showed uniform gel-like glue; and then, the gel-like egg glue was filled into syringe to test injectability.

Furthermore, two groups of samples with same concentration were prepared for rheology test. At first, 0.875g air-dried egg albumen powder was dissolved into 1ml double deionized water and stirred by using a spoon to obtain uniform viscous EAA glue; secondly, urea-mixed egg albumen was prepared by dissolving 0.875g EAA into 8M urea solution and also stirred until fully dissolved.

3.5.2 Injectability test

Injectability test was used to asses the formability and injectable behavior of EAA. The test was based on a 30ml-syringe with syringe needle (gauge 19, inner diameter of needle is 0.7mm).
3.5.3 Rheology test

Rheology tests were used to evaluate the adhesive behavior and viscoelasticity of EAA and urea-mixed egg albumen. Rheology frequency sweeping test was performed on the TA DISCOVERY HR1 hybrid rheometer, with a cone substrate (20mm diameter, 2° angle, steel). The strain was set up as 0.2%, and frequency increased from 0.1rad/s to 100 rad/s by steps.

3.6 Shear Adhesion Characterization

3.6.1 Shear strength test on machine

3.6.1.1 Sample preparation

Egg albumen adhesive with concentration of 0.875g/ml, which was made from 70mg egg albumen powder and 80ul double deionized water for total 200ul, was coated onto different substrate, including glass, PDMS, pigskin tissue; then, two same substrates were overlapped over each other. Two commercial glues, cyanoacrylate medical adhesive glue and medical fibrin glue, were used as manufactures’ instruction to prepare 200ul onto each substrates group.

Glass substrates were used as given (purchased object glass slide with dimension 75mm × 25mm), PDMS substrate was prepared by curing of SYLGARD®184 silicone elastomer at 80°C. Pigskin tissue substrates were freshly prepared by thawing the stored bulk product at room temperature for 1h, and hairs and fat parts were removed from the tissue before test. Both PDMS and pigskin tissue substrates were
cut to a rectangular shape of 75mm × 25mm.

3.6.1.2 Shear strength test

The shear strength ability was then conducted by applying the universal tensile tester (INSTRON 5965). Three groups of samples were tested on three different substrates. These three groups are egg albumen adhesive, cyanoacrylate medical adhesive glue, and medical fibrin glue. These three substrates are polydimethylsioxane (PDMS), glass slides, and pigskin tissue. The dimensions of all substrates were used as 75mm × 25mm, and the applied overlapping area on these substrates was 25mm × 20mm. EAA powder and water were mixed homogeneously with using of a spoon at the ratio of 0.875g EAA / 1ml water. Prepared EAA adhesive of ~200µl was casted onto a piece of substrate and covered with another piece of substrate under moderate pressure. Similar amounts of commercial adhesives were casted on control groups for comparison. The curing time for all samples is 5mins at room temperature under moderate pressure. All groups’ samples were tested by utilizing a 500N load force with a test rate of 10 mm/min with gauge length 5cm, except the group of EAA-glued glass slides substrates, which was tested by using a 5000N load cell. The load-extension parameters were given by instrument software. Each group was tested three times by controlling identical setup and size. The results were figured by using mean values and standard derivation.
3.6.2 Shear strength test by lifting heavy objects

3.6.2.1 Sample preparation

The dimensions of glass substrates were used as 75mm × 25mm, and the applied overlapping area on these substrates was 25mm × 20mm. Prepared 0.875g/ml EAA adhesive of ~200µl was casted onto a piece of glass substrate and covered with another piece of substrate under moderate pressure for 5 mins. Then, one end of glass substrate was bonded by 3M adhesive tape with heavy objects (6kg).

Pig bones were purchased from local market store, and broke them at middle point by pincher, the cross-section area of fractured bone was measured as 14mm × 9mm. Then prepared 0.875g/ml EAA of 50µl was coated onto fracture area uniformly, bone was bonded with heavy objects (1.5kg) after that.

3.6.2.2 Shear strength test

One end of bonded glass substrates and broken bone was held and lifted upward straightly, respectively; while, the other ends were bonded with heavy objects. For the test on glass substrates, heavy objects were lifted from table and held in air for 30s without relative slippery between EAA-glued area; and 10s for fractured bone test.

3.6.3 Underwater shear strength test

The dimensions of glass substrates were used as 75mm × 25mm, and the applied overlapping area on these substrates was 25mm × 20mm. Prepared 0.875g/ml EAA
adhesive of ~200µl was casted onto a piece of glass substrate and covered with another piece of substrate under moderate pressure for 5 mins. Then, a thin layer of Vaseline was daubed uniformly on all edges to enclose the gaps between two glass plates; sample was then immersed into water for 1, 2, and 3 days. EAA glued glass substrates was lifted 6kg and 5kg objects for shear strength test after that.

3.7 In vivo Evaluation

3.7.1 Wound healing test in vivo

All animals tested in the research were purchased from Nanjing Medical University Experimental Animal Center. Also, the operation procedures of animal tests were permitted by Animal Care and Use Committee of Nanjing Medical University. Six male SD rats (200-250g) were anaesthetized with chloral hydrate (300mg/Kg). Three incisions (2 cm) were made on the back of animals after sterilization. Each randomly received different kinds of closure, which is either suture, hemostasis only or adhesion with egg glue. 4-0 unresorbable suture was used to close the wound. The wound incision was covered with PCL electrospun membrane coated with 50 mg EAA. All egg albumen powder and PCL nanofibrous membranes were sterilized under UV exposure for 3 hours before applying on animals.

3.7.2 Hematoxylin and eosin staining and analysis (H&E)

After 5-day post-operation, each rat was sacrificed and three 2 cm × 1cm rectangle piece of skin containing the wound was resected. Samples were then immobile in
concentration of 10% neutral-buffered-Formalin solution for 24 hours. The middle parts of scar was then stained via hematoxylin-eosin (H&E) and was evaluated using an optical microscope.

3.7.3 Degradation of implanted egg albumen *in vivo*

EAA was injected subcutaneously under the middle dorsal regions of the skin in rats, and EAA was harvested at 7, 21, 35 days post-operation, respectively; after that, rats were sacrificed and corresponding tissues were resected and stained with H&E stain and Masson trichrome stain, respectively.
Chapter 4 Results and Discussion

4.1 Introduction

This chapter introduces the results and discussions of experiments. The FTIR-ATR, Optical microscope, and SEM results are shown in Section 4.2. The Mechanical characterization, including shear strength evaluation on machine and underwater shear strength test, is also discussed in Section 4.4.

4.2 Characterization of Egg Albumen’s Chemical Properties

4.2.1 FTIR-ATR characterization

FTIR-ATR spectra of air-dried egg albumen powder and EAA-water adhesive was obtained to analyze the chemical structures and the mechanism of egg white albumen adhesive.

From the FTIR spectra as shown in Figure 2A, 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.

Furthermore, based on the secondary structure analysis of EAA glue (0.875g dry powder/ml water) in Figure 2C, dry egg white albumen showed typical specific
Figure 2. (A) FTIR-ATR spectra of EAA powder and EAA-water adhesive, (B) FTIR-ATR spectra of secondary structure analysis of dry egg albumen powder from amide I region, (C) FTIR-ATR spectra of secondary structure analysis of EAA-water adhesive from amide I region.
characteristic peaks of protein/polypeptide, including the strong wide-broad peak at 3275 cm\(^{-1}\) caused by N-H stretching, the strong sharp peak at 1636 cm\(^{-1}\) from the C=O stretching vibrations (amide band I, a sign of antiparallel \(\beta\) sheet stabilized by intermolecular hydrogen bonds), the sharp peak at 1515 cm\(^{-1}\) (amide band II) due to both C-N stretching and N-H bending vibration, and amide band III at 1230 cm\(^{-1}\) [164]. 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. As well, 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 1). 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 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[165]. Compared with same concentration egg white albumen-water mixture, the egg white albumen-8M urea solution mixture lost its good viscoelasticity and high adhesion (as shown in Figure 8).[166]
Table 1. The secondary structure fractions of EAA glue

<table>
<thead>
<tr>
<th>Peak position</th>
<th>Area (%)</th>
<th>Assigned secondary structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1615.1</td>
<td>2.44</td>
<td>β sheet</td>
</tr>
<tr>
<td>1624.5</td>
<td>21.23</td>
<td>β sheet</td>
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<td>1636.4</td>
<td>28.13</td>
<td>β sheet</td>
</tr>
<tr>
<td>1649.9</td>
<td>14.21</td>
<td>unordered</td>
</tr>
<tr>
<td>1657.7</td>
<td>15.51</td>
<td>α helix</td>
</tr>
<tr>
<td>1671.2</td>
<td>7.34</td>
<td>β turn</td>
</tr>
<tr>
<td>1682.4</td>
<td>7.97</td>
<td>β turn</td>
</tr>
<tr>
<td>1695.2</td>
<td>3.16</td>
<td>antiparallel β sheet</td>
</tr>
</tbody>
</table>
The mechanism of egg white albumen adhesive was interpreted in Scheme 1. From the view of primary structures, native egg white albumen generally is comprised of ~90% water and ~10% proteins, which includes ovalbumin (~54%), conalbumin (~12%), ovomucoid (~11%), and globulins (~8.0%), et.al. [167-169] The covalent bonding or chemical reactions did not play a critical part 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 native 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 (15.5%))[170]. 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.[166]
Scheme 1. The schematic mechanism interpretation of pressure sensitive egg white albumen adhesive (EAA)
While, the secondary structures of dried egg white albumen were characterized by analyzing the amide I region peak of FTIR-ATR spectra [170, 171]. Raw spectra peaks were self-deconvolution to overlain the single peaks, in this way, 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 α-helix and unordered conformation dropped to 14.8% and 17.0% respectively, whereas the β-sheet and β-turn jumped to 48.2% and 20.0% dramatically (see Table 2), indicating high degree of inter/intra molecular hydrogen bonding interaction between different protein backbones that leaded 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[172]. 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[173].
Table 2. The secondary structure fractions of dry egg white albumen powder

<table>
<thead>
<tr>
<th>Peak position</th>
<th>Area (%)</th>
<th>Assigned secondary structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1615.7</td>
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<td>1682.4</td>
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<td>β turn</td>
</tr>
<tr>
<td>1694.6</td>
<td>8.15</td>
<td>antiparallel β sheet</td>
</tr>
</tbody>
</table>
4.2.2 Transmittance visible spectra

The recombinant egg white albumen solution was prepared by mixing dried egg white albumen powder with deionized water at the concentration of 10% (similar to raw egg white albumen solution), which was dispersed in ultrasonic bath at ambient condition for 4h. Compared with raw egg white albumen, the recombinant egg white solution looked opaque and only had the transmittance of ~0%, but the raw egg albumen solution had the transmittance of higher than ~60%, suggesting the protein network crosslinked by hydrogen bonds could not be dissolved reversibly, as shown in Figure 3B.

Moreover, fresh egg albumen was shown in Figure 3A (right) was more visibly transparent than the recombinant egg albumen solution obviously, which again proved that the recombinant egg white solution could not be dissolved reversibly.
Figure 3. (A) Photograph of recombined egg albumen solution from dry albumen powder (left) and native egg albumen solution from fresh egg (right), and (B) the Transmittance visible spectra of raw egg white albumen liquid and 10% recombinant egg albumen solution.
4.2.3 Surface topography characterization

4.2.3.1 Optical microscope topography

Optimal microscopy technique revealed the differences in the surface topography between EAA and natural fresh egg albumen liquid. The appearance of EAA showed as gel-like (Figure 4), which is non-homogenous; while the appearance of fresh egg albumen is more homogenous. Also, this topography results showed that the recombinant egg white solution could not be dissolved reversibly, and the egg white albumen proteins polypeptides crosslink networks were swollen rather than dissolved.
Figure 4. Optimal microscope images of EAA.
4.2.3.2 Scanning electron microscopy topography

Furthermore, the technique of scanning electron microscopy was applied to evaluate and provided the surface microstructure of EAA coated on PCL nanofibrous membrane; the SEM image showed that the separated albumen powder was mixed and integrated together to a whole piece of gel after stirring with water (Figure 5). The SEM image again exhibited EAA’s non-homogenous character.
Figure 5. SEM image of EAA coated on PCL nanofibrous membrane.
4.3 Adhesive Behavior Evaluation

4.3.1 Injectability assessment

As one of important characters, injectability can be used to assess the adhesive behavior of a glue since viscosity is the necessary factor that a glue should have. For our egg albumen glue, concentration is the only way to affect its viscosity. Therefore, in order to evaluate and obtain best concentration for adhesive and injectable behavior, the egg albumen glue was first prepared at 3 different concentrations, 1.400g egg powder/ml water, 0.875g egg powder/ml water, and 0.636g powder/ml water. Among them, 1.400g is the maximum amount of egg powder could be dissolved in 1ml water, it shows good adhesive but could not be injected through a syringe needle no matter what size of the needles because 1.400g/ml concentration egg glue has no liquid mobility. While the concentration of 0.636g/ml egg glue could be injected from needle, but it has poor viscosity. Finally, the EAA at concentration of 0.875g powder/ml water showed the best injectability from a syringe needle (gauge 19, inner diameter of needle is 0.7mm) with great viscosity, formability and adhesive ability (Figure 6). As shown in Figure 6B and 6C, EAA could be pinged like glue between two fingers, and injected from needle with continued line. Those great injectable behaviors lead EAA with concentration of 0.875g powder/ml water has been chosen for further experiments.
Figure 6. Egg albumen adhesive: (A) Egg albumen (0.875g/ml); (B) EAA pinned between two fingers (0.875g/ml); (C) The adhesive is injectable and shows adhesive-induced long consistence from the medical syringe needle (0.875g/ml).
4.3.2 Rheology test evaluation

In order to further evaluate the viscosity and adhesive behavior of designed EAA, the rheology test was applied Figure 7 stated the complex viscosity (Pa.s) versus angular frequency (rad/s) of EAA with concentration of 0.875g/ml. The complex viscosity was around 150000 Pa.s at the beginning angular frequency point 0.1rad/s, and then the complex viscosity went up to the maximum value of 280000 Pa.s at 0.3 rad/s; after that, the complex viscosity decreased as angular frequency increased. While the relationship is linear, so, it can be concluded that the complex viscosity of EAA decreases with growing shear rate, so this result indicated a typical shear-thinning behavior. This shear-thinning behavior proved the 0.875g/ml EAA has the adhesive and injectable ability, which is in concert with Figure 6C for the injectability.
Figure 7. Shear-thinning behavior of EAA.
Furthermore, compared with same concentration, the rheology evaluation related to storage modulus (Pa) Vs. frequency sweeping of 8M urea doped EAA (0.4375g egg albumen powder / ml 8M urea solution) and water mixed EAA (0.4375g egg albumen powder / ml water) have been conducted. From Figure 8, it showed that 8M urea doped EAA lost its good viscoelasticity and high adhesion, which indicated a much lower storage modulus since the strong hydrogen bond network broken by urea in egg white albumen-water mixture; however, the same concentration water mixed EAA showed great storage modulus increasing with frequency sweeping inversely.
Figure 8. Profiles of frequency sweeping tests.
In this frequency sweeping rheology test, the concentration that used is 0.4375g/ml, instead of 0.875g/ml; because egg albumen – 8M urea solution mixture sample had too poor adhesion, and could not adhere to a whole piece of gel for rheology frequency sweeping test at all. Otherwise, even the lower concentration EAA could showed better viscoelasticity and higher adhesion than urea, the best concentration would obtain much better results.

**4.4 Shear Adhesion Characterization**

Schematic illustration indicated the super adhesive behavior of the treated egg albumen and the medical application with PCL nanofibrous (Scheme 2). Raw egg albumen was originally taken from fresh eggs; and then, showed adhesive by stirring uniformly with double deionized water. The great adhesive ability could bear 6kg heavier. In addition, by combined with PCL nanofibrous, it showed good biocompatibility and treatment effect for skin incisions closure on rats.
Scheme 2. Schematic illustration of egg albumen adhesive (EAA) showing remarkable adhesive strength and EAA coated PCL nanofibrous for skin incisions treatment.
4.4.1 Shear strength evaluation on machine

For purpose of evaluating the shear adhesive ability of EAA, the lapse shear strength tests were conducted. By comparing with two other medical glues- cyanoacrylate medical adhesive glue and medical fibrin glue, adhesive strength of those glues was conducted on different substrates: polydimethylsiloxane (PDMS) substrates, glass slides substrates, and pigskin tissue substrates. The reason to choose these three substrates is that PDMS showed hydrophobic which could be act as hydrophobic substrates, glass slides are hydrophilic materials which could show as hydrophilic substrates, while pigskin tissue could represent human skin somehow; otherwise, those two commercial medical glues are now popular used medical sealant. The setup is briefly shown as two substrates were glued to each other by overlapping an area through all different adhesive glues (Figure 9D), the EAA concentration here used is 0.875g/ml.
Figure 1. Shear adhesion stress-strain curves of EAA, cyanoacrylate synthetic adhesive and fibrin glue on 3 different substrates, including (A) PDMS substrates, (B) glass substrates, and (C) pigskin tissue substrates. (D) Photograph of prepared glue cured pigskin substrates for shear adhesion test (adhesion area is 25mm×20mm for all samples).
Based on test results (Figure 9A, B, and C), EAA exhibited huge differences on different substrates. On hydrophobic PDMS substrates (Figure 9A), the maximum adhesive shear strength was found as 5.3±1.1KPa by using EAA, whereas for cyanoacrylate glue this number was 1.8±0.4KPa, and 0.5±0.2KPa for fibrin glue. Commercial cyanoacrylate glue is one of the strongest medical adhesive glue now used for wound closure, even it would be problem sometimes when surgeons operated cyanoacrylate glue during surgical, because it is hard to remove from the surgical equipment if unproperly operation happened; however, EAA showed about three times higher adhesion. Furthermore, according to Figure 9B, remarkably, the adhesive shear strength on hydrophilic glass substrates could surprisingly reach up to a maximum of 216±80.4KPa for EAA, but the shear strength was less than 3.0KPa for rest of the two commercial glues; the adhesive ability of EAA is more than 60 times higher than any other two commercials. Therefore, EAA showed more excellent adhesive behavior on both hydrophobic and hydrophilic substrates than commercial cyanoacrylate and fibrin glue.

Finally, as shown in Figure 9C, the tests were conducted on animal tissue - pigskin tissue, the highest adhesive shear strength on pigskin could reach up to 56.2±15.2KPa for EAA, while the maximum shear strength is 55.4±16.9KPa, the results indicate that EAA could offer even reliable adhesion as the most commonly used commercial cyanoacrylate medical adhesive glue; and much better than fibrin glue (more than two times higher), whose maximum shear strength is 24.0±9.3KPa. Based on those test data, 0.875g/ml EAA provided reliable adhesion and showed potential commercial
applications; while EAA gained its advantages of extra low cost and simple handle method.
4.4.2 Shear strength test by lifting heavy objects

Figure 2. Lifting heavy stuffs test of EAA glued substrates. (A) EAA glue enduring a weight of 6kg (two 500g small objects on the top, and one 5kg object on the bottom, right down corner showed the 20mm × 25mm overlapped area on glass substrates); (B) The glued broken bones lifting up to 1.5kg heavy objects (the cross section area of fractured bone is 14mm × 9mm, shown in right corner).
Since EAA showed super high adhesion on glass substrates, the adhesion capacity of EAA on glass substrates should be further evaluated straightforwardly, as shown in Figure 10A. EAA glued glass substrates were then used to lift heavy objects, and exhibited incredibly strong shear adhesion. A small adhesion area of 25mm × 20 mm could afford a huge gravitational force of 6 kg steadily for 30s. The calculated shear adhesion strength of EAA on glass was up to 117.7KPa, which was even greater than the previously reported adhesive materials for glass substrates. For example, Yuk et al developed an double-network hydrogel with exceptional adhesive property to bond two glass plates together (dimension: 50mm × 50mm × 1.5mm), which could lift up and hold a 25kg object, the calculated shear stress of their adhesive hydrogel was 98.1KPa[174]. Comparatively, our EAA glue displayed a higher adhesion capacity and could be achieved at extra low cost and simple synthesis method. This bonded glass test proved EAA gained outstanding adhesion again in a very straight and visible way.

As one of tissue, bones adhesion is also a valuable examination to test EAA’s adhesive ability. We then tested EAA on broken bones, the EAA was also exhibited interesting adhesion performance. A fractured bone with fracture area of 14mm × 9mm was glued together with 0.875g/ml EAA, and then lifted the heavy objects weighing up to 1.5kg for 10s. It could afford a gravitational force, and the adhesive strength applied could be reached to 116.8KPa (Figure 10B). EAA showed excellent potential application on not only the pigskin tissue, but also the bones.
4.4.3 Underwater shear strength evaluation

Internal human environment is a liquid based environment, therefore, in order to apply any medical glues or adhesives inside human body, the water proof or water resistance tests would be necessary. In this way, the underwater performance of EAA was also evaluated for further potential application in wet environment. Sealed with Vaseline, the EAA also showed exceptional wet adhesive strength. The EAA glued glass substrates were immersed in water up to 3 days, and a thin layer of Vaseline was daubed on all edges to enclose the gaps between two glass plates. After 2 days, the adhesion area was still able to afford a force of 6kg for 30s, which is also 117.7KPa (Figure 11). After 3 days of immersion in water, the affordable force of the glued glass plates slightly dropped to 5kg, maintaining up to 83% of its adhesion capacity underwater. Since Vaseline is a neutral product that widely used in daily makeup like lotions and creams, it showed no harmful for human; so, EAA could have the potential used inside human body by combined with Vaseline.
Figure 3. Underwater adhesive strength of EAA: glued glass substrate after exposure to water lifting the heavy objects.
4.5 In vivo Evaluation

4.5.1 Wound healing test in vivo

Finally, to evaluate its adhesive property in vivo, a rat model was used to study the effects of EAA coated PCL nanofibrous sheet on the wound site. The steps are simple and easy to be operated by surgeons. All egg albumen powder and PCL nanofibrous membranes were sterilized under UV exposure for 3 hours before applying on animals. With EAA coating on, PCL nanofibrous membrane was placed on the top of the incision area for wound site repairing. It was observed that the wound site was adhered after only five minutes, and thereafter left for healing investigation (the process was shown in Figure 12A-D).
Figure 4. Egg albumen adhesive accelerating wound closure. A, B and C: Steps employed during the surgery to apply EAA coated PCL nanofibrous membrane (a), suture (b) and blank control group (c) on the wound sites; (A) Three incisions (2cm) were medical cut on the back of mice after sterilization; (B) and (C). EAA coated PCL nanofibrous membrane (a) was covered on the incision; (D) wound site after 5 minutes of applying EAA. (E) Three incisions made on the back of rat after 5 days post operation.
After 5 days, all the three wound sites were examined to gain an insight on the healing process and to monitor the changes in the wound enclosure (Figure 12E). The wounds operated by medical suture (b) and glued by EAA (a) were found as more reliable healing as compared with the control group, which was left open without any treatment for hemostasis only. After 5 days post-operation, EAA site presented excellent property of wound closure, and no evidence of infection or inflammation was apparent. Wounds closed by suture recovered completely at 5 days postoperatively. However, the blank groups showed dehiscence (Figure 12E). Therefore, the PCL coated with EAA results in wound healing in a same manner as that of conventional suture.

4.5.2 Hematoxylin and eosin stained analysis (H&E)

Histopathological studies were conducted to study the effects of wound healing in vivo. The incisions treated with the EAA had longitudinal collagenous fiber, sporadic neutrophils and fibroblasts beneath the interfaces. Epithelium was also consecutively integrated with basement membrane and no deep openings were observed in the tissues. Moreover, hair regrew was observed across the incision without scarring. Thus, EAA promoted the overall wound healing (Figure 13A, D). However, in the case of sutured wounds, less anomalous collagen fibers and more neutrophils and fibroblasts (Figure 13B, E) and hairless wound site were found as compared with the ones found in EAA treated tissues. The incision treated with hemostasis was found in filling up with large amounts of granulation tissues, and contented mass of polymorph
nuclear leukocytes, macrophages, fibroblasts and blood capillaries were found in the tissues (Figure 13C, F). Therefore, the incisions treated with EAA got better recovering as compared to the tissues treated with conventional sutures. [166]
Figure 5. Histopathological evaluation: (A) and (D) Treated with EAA; (B) and (E) Treated with suture; (C) and (F) Hemostasis only for blind control group (100×).
4.5.3 Degradation of implanted egg albumen in vivo

The egg albumen was degraded with time and still visible after 35 days of implantation. At 7 days after implantation, the peaceable acute inflammatory reaction was observed in outmost layer with the presence of representative multinuclear cells; the roughness tissue with fibroblast propagation and few loose collagen layer construction were showed around the egg albumen (Figure 14A, D, G). After 21 days, egg albumen began to lose structural integrity and almost filled by invading cells, the inflammatory response decreased with the disappearing of the leukocytes and increasing of macrophages (Figure 14B, E), also the thin fibrous capsule secluded the albumen from native tissue by rejection reaction of foreign body, which including some macrophage corroding the albumen surface, a collagen encapsulation consisting multilayers fibroblasts (Figure 14H). The host response against of the EAA was becoming slight with growing implanted time. Moreover, vascularization was observed in the periphery fibrous tissue. At 35 days after implantation, more mature vessels could be observed around encapsulated smaller pieces of degraded gel (Figure 14C, F), a thicker fibrous capsule was formed around the whole implant and surrounded collagenous connective tissue (Figure 14I).
Figure 6. Degradation of subcutaneously implanted egg albumen in rats. Egg albumen was harvested at 7, 21, 35 days postoperation and stained with HE (A-F) and Masson trichrome stain (G-I), respectively. Bars in (A-C), (G-I) =200μm and (D-F) =50μm.
Chapter 5 Summary and Conclusions

In summary, EAA glue from fresh eggs can be fabricated via the simple processes of air-drying, grinding and mixing with proper amount of water, and the novel egg albumen adhesive was an economical and environmentally friendly biomedical glue. The glue is versatile and could be coated onto PDMS, glass, and skin. Compared with other two types of popular commercial medical glues, egg albumen adhesive showed remarkable adhesive strength, especially outstanding shear adhesion performance on glass substrates. As well, EAA could be promising medical glue for bone fracture closure due to its excellent performance on broken bone adhesion. Surprisingly, it showed great underwater adhesive, and then this excellent underwater adhesion promised the further potential application inside human body.

The EAA is also biocompatible, biodegradable, and easy to operate for wound site repairing based applications. On rats wound site closure, EAA coated PCL nanofibours membrane showed much better wound repairing ability compared to traditional medical suture, and did not show strong long-term inflammatory response in vivo subcutaneous implantation degradation experiment.

Therefore, considering its abundant source, simple and environmental friendly fabrication process, inherent non-toxicity, biocompatibility, and extra low cost, EAA could be a medical adhesive candidate with brilliant perspective in clinic medication.
Chapter 6 Suggestions for Future Work

1. Generally, egg caused allergy affects 1-2% children through the world base on some reported literatures. Although the in vivo skin adhesion and subcutaneous implantation tests in rats did not show significant immune-response in this research, it should be cautious while the EAA is applied to human body. Therefore, a further research should be conducted for its potential immunogenicity in future work.

2. Since the EAA showed great adhesive behavior even under water for 3 days, it exhibits potential application for wound closure in body such as gastric, hepatic, hernia wound seal; so, the in vivo tissue wound closure ability would be assessed in future research.

3. Egg albumen adhesive has good injectability through the syringe needle, the potential application for 3D printing would be researched in further work.
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