# Preparation of a mussel-inspired bio-adhesive composed of polylysine modified gelatin and 3,4-dihydroxybenzaldehyde

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## Abstract

Bio-adhesion has been wildly used in various biomedical applications, such as orthopedics, surgery, drug delivery systems, etc. While preparation of bio-based adhesives with high strength and biocompatibility is still a major problem. Gelatin is a promising material for bio-adhesive due to protein's abundance, its excellent biocompatibility, resorbability and typically low immunogenicity. However, its poor mechanical properties and poor adhesion strength limits its applications. Inspired by the mechanism of bio-adhesive proteins such as mussels and oysters, we used a two-step process to develop a gelatin-based adhesive. First, polylysine (ε-PL) was introduced into gelatin chains catalyzed by microbial transglutaminase (MTG) to improve the number of amino groups available for the next reaction. Second, 3,4-dihydroxybenzaldehyde (DHBA) was intercalated to form a gelatin-polylysine-benzaldehyde (Gel-PL-DB) adhesive. The as-prepared adhesives were characterized by ultraviolet-visible spectroscopy (UV-vis), infrared spectroscopy (FTIR), and thermogravimetric analysis (TGA/DTA). The results of UV-visible spectroscopy showed that ε-PL was successfully grafted onto gelatin molecules, and FTIR test results indicated that DHBA was successfully polymerized into Gel-PL. Adhesive strengths were evaluated using fresh porcine skin as a model system, which was lapped under constant temperature and humidity for 9 h. The highest adhesion strength was 55.14 ± 6.51 Kpa, which was 10 times that of Gel-PL. In summary, the bio-based tissue adhesives synthesized in this study have good biocompatibility, high adhesion properties, and has good application potential in the biomedical applications.

Keywords: Gelatin-based adhesive; Mussel-inspired; High adhesion properties

## 1 Introduction

In clinical medicine, the damage of skin, stomach, heart and other tissues and organs as well as surgery needs to be repaired and treated. Although traditional surgical sutures, suture nails and other repair methods can effectively close the wound and prevent the extravasation of blood and tissue fluid[1], However, these traditional invasive wound closure methods may cause damage to surrounding tissues, damage tissue nerves, and increase patient suffering[2, 3]. At the same time, which increase the risk of wound infection and tissue foreign body reaction[4]. Therefore, bio-adhesive which can partly replace suturing in clinic to join tissues together has become a hot topic in the field of biomedicine [5]. At present,

the developed bio-adhesives mainly include synthetic polymer adhesives with cyanoacrylate, polyurethane and polyethylene glycol as the main raw materials and natural polymers such as protein and polysaccharide represent good raw materials [3]. Synthetic polymers have various functional groups and can achieve high mechanical strength [6]. The synthesized bio-adhesives are relatively stable in structure and usually have high adhesion and good mechanical properties. Generally speaking, the potential toxicity induced by monomers and crosslinking agents, poor biodegradability and biocompatibility and poor patient compliance limit the practical applications of synthetic polymer bio-adhesives.

Accordingly, natural polymers such as gelatin, chitosan, and hyaluronic acid, which usually have good biocompatibility and degradability, have been explored as alternative materials to invent new bio-adhesives [7]. Among them, gelatin is most widely used in the medical field, due to its non-immunogenic, non-antigenic and harmless metabolic by products upon degradation inside the body [8]. In addition, gelatin share the same primary structure with collagen, which may facilitate cellular adhesion and proliferation [9]. However, due to its high degree of hydrophilicity and swelling rate, native gelatin exhibits weak bio-adhesive properties [10]. The surge of interest in the enhancement of bio-adhesive properties of gelatin has encouraged various efforts, such as blending gelatin with other polymers and chemical modification [11]. These efforts aim to establish stronger interactions between gelatin chains and other polymers, or between gelatin chains and tissue substrates at the molecular scale to improve their mechanical properties and adhesion.

In recent years, inspired by mussels, many researchers have conducted extensive research on the application of catechol and its similar organisms in bio-adhesives, and developed catechol bio-adhesives with both waterproof and adhesive properties [12]. Catechol groups enable adhesion through various covalent and noncovalent bonds between gelatin chains and tissue substrates [13,14]. Covalent adhesion is driven primarily by oxidation of catechol to reactive o-quinones. And the resulting o-quinones subsequently react with nucleophiles (e.g., amines and thiols) via Schiff base formation, Michael type addition, and dicatechol coupling [15]. Lee et al [16]. used dopamine self-polymerization on a variety of materials to form a thin and surface-attached dopamine film, which can be manufactured or deposited by secondary reaction to form a multifunctional polymer coating.

In this paper, a bio-adhesive (Gel-PL-DB) for injectable closure of tissue trauma was studied. The DOPA-like structure (bisphenol structure) 3,4-dihydroxybenzaldehyde was introduced into the gelatin chain by Schiff base reaction to produce π-π interaction [17] and enhance cohesion. Prior to this, gelatin was grafted by poly-lysine using microbial transglutaminase (mTG) to improve the reactive sites for 3,4-dihydroxybenzaldehyde. The application of enzymatic crosslinking ensures the non-toxic and biocompatibility of the as prepared bio-adhesive.

## 2 material and method

### 2.1 material

Gelatin (Type A from porcine skin) was obtained from Sigma-Aldrich. ε -Poly-L-lysine(ε-PL), 3,4-dihydroxybenzaldehyde (DHBA) were purchased from Shanghai Macklin Biochemical Co., Ltd.

microbial transglutaminase (mTG) was purchased from Shanghai Yuanye Biotech. Co., Ltd.

### 2.2 Preparation of Gelatin-polylysine/DHBA (Gel-PL-DB) adhesive

A certain mass of gelatin was dissolved in deionized water at 50 °C, and then different percentages of -polylysine (calculated by gelatin mass) were added respectively. After successive mixing, the crosslinking reaction by mTG due to their high content of lysyl residues was carried out at 50 °C for 12 h. Subsequently, the solution was dialyzed against distilled water 10 KDa molecular weight cut-off membrane, and freeze-dried for subsequent using.

The above freeze-dried powder (Gel-PL) was redissolved in distilled water a concentration of 2.5 %, and DHBA with different percentages (calculated by Gel-PL mass) was added. The reaction was stirred at 37 °C for 12 h, and then placed in a dialysis bag with a molecular weight cut-off of 8-14 KDA for dialysis. Gel-Ply-DB samples were prepared after freeze-drying.

### 2.3 Determination of free amino content of Gel-PL

The free amino content of Gel-PL samples was determined by o-phthalic aldehyde (OPA) method reported by Wang [18]. Briefly, a mixed solution was prepared by dissolving 3.81 g of sodium tetraborate decahydrate and 100 mg of sodium dodecyl sulfate (SDS) in 75 mL of deionized water. Then, 80 mg of o-dibenzaldehyde (OPA) was dissolved in 2 mL of ethanol. After mixing the two solutions, 88 mg of dithiothreitol (DTT) was added and diluted to 100 mL with deionized water. Gel-PL samples were prepared into 1 mg/ml aqueous solution respectively. For determination, 400 μL of the sample solution was mixed with 3mL of OPA reagent and reacted at 35°C for 2 min, and the absorbance was measured at 340 nm using an ultraviolet-visible spectroscopy (NanoPhotometer NP80 Touch, Germany).

### 2.4 Determination of Zeta potential of Gel-PL solution

The determination of the Zeta potential is commonly employed to estimate the surface charge density of biomolecules. The analyte solution was prepared by diluting 1 mg Gel-PL to 10 mL water, and adjusting to pH 7.0. Apparent zeta potentials were measured with a Malvern laser particle size analyzer (ZEN3600, UK).

### 2.5 Characterization of Gel-Ply-DB

In this work, the synthesized samples have been characterized by UV-Vis and FT-IR spectroscopy techniques as well as TGA analysis.UV-vis spectrum was carried out with a Shimadzu (UV 2600) in the range of 200 to 900 nm. The FT-IR spectrum was taken in the range of 4000-400 $cm^{-1}$ using a S™10 FT-IR spectrometer (Thermo Nicolet) with ATR mode.

Freeze drying power of Gel-PL-DHBA was placed in thermogravimetric analyzer (TGA, Mettler-Toledo) for thermal degradation analysis. The weight loss at 50-800 °C was measured by TGA in nitrogen atmosphere. The scanning rate was 10 °C / min and the nitrogen flow rate was 60 mL / min.

## 2.6 Adhesion strength test

The adhesion strength of gelatin-based adhesive (Gel-PL-DHBA) was determined according to ASTM F2255-05 [19] standard. The 75 × 25 mm fresh pig skin was used as the substrate. The freeze-dried Gel-PL-DHBA was configured into an aqueous solution with a mass fraction of 20 %, and the magnetic stirring was performed at 50 °C until it was completely dissolved. The 100 ul test solution was evenly applied to the surface of fresh pig skin by using a pipette gun or 1 ml disposable sterile syringe for overlapping adhesion. The length and width of the overlapping surface were both 25 mm, and the thickness was controlled within 2 mm. The lapped fresh pigskin was cured at room temperature, and then the bonding strength was tested by a servo-controlled desktop tensile tester (GOTECH, AI-3000-U, Taiwan Province).

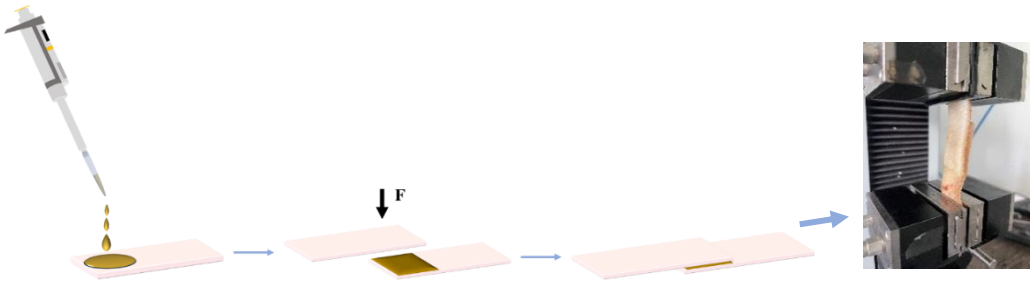


Figure.1 Procedure for testing the bond strength of fresh pig skin.

## 3 Results and Discussions

### 3.1 Structural analysis of Gel-PL

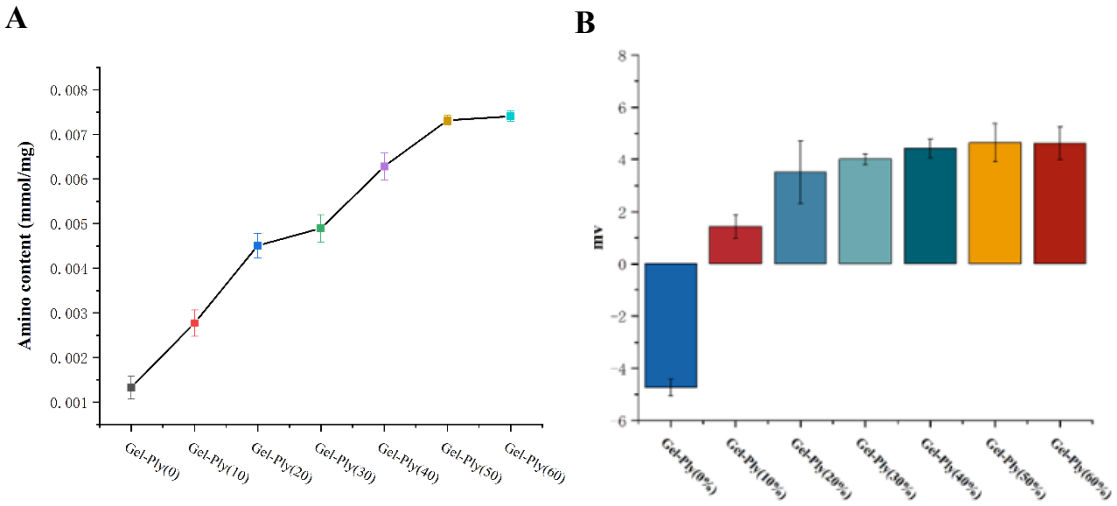


Figure.2 The free amino content (A) and of Zeta potential (B) Gel-PL samples.

The free amino content of Gel-PL can be used to evaluate the degree of ε-PL grafting on gelatin [20]. As shown in the figure.1A, with the increase of ε-PL amount, the free amino content of Gel-PL

increases continuously. It indicated that ε-PL was grafted successfully on the gelatin chains. Nevertheless, the free amino is no longer increasing when the amount of ε-PL reaches 50%. This may be due to the fact that the binding site (Gln in gelatin chains) was saturated by ε-PL. This significantly improved number of free amino provides sufficient active sites for the inducing of DHBA.

Under neutral condition, the Gel-PL molecules were positively charged because of the protonation of the free amino groups, which can be detected by Zeta potential measurements [21]. The results are shown in the figure.1B. As expected, the test results are consistent with the pattern presented in the determination of free amino. Compared with pure gelatin, the voltage of Gel-PL samples was positive, and with the ε-PL amount increasing, the Zeta potential increases continuously. When ε-PL amount is 50 % of the gelatin mass, the voltage reaches the maximum.

### 3.2 UV-Vis spectroscopy

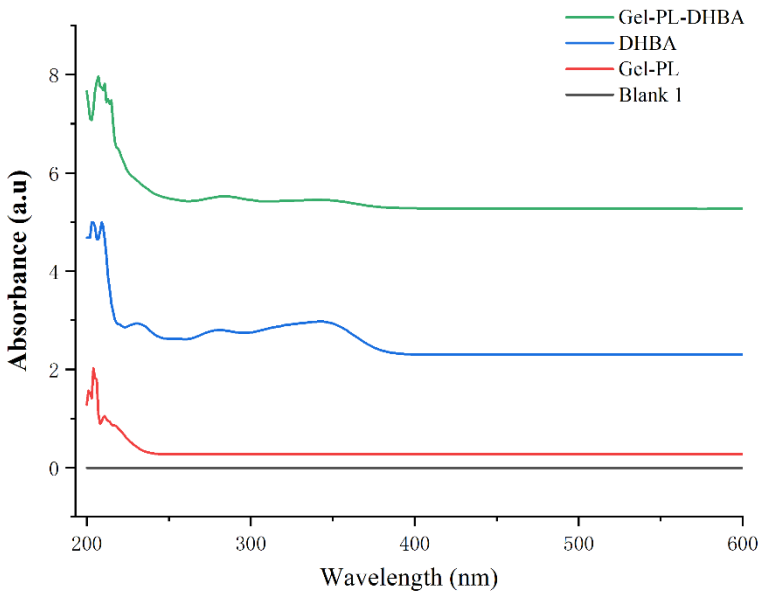


Figure.3 Uv-vis spectra of Gel-PL, DHBA and GEL-PL-DB.

The samples of Gel-PL, DHBA and Gel-PL-DB were determined by UV spectrophotometer. As shown in the figure.2, clear absorption peaks at 280 nm and 350 nm could be found in the samples of DHBA and Gel-PL-DB, which originate from the benzene ring and bisphenol structure contained in DHBA [22]. Meanwhile, Gel-PL has no obvious absorption peaks at the both wavelengths, indicating that DHBA has been successfully grafted into the Gel-PL.

### 3.3 FT-IR spectra

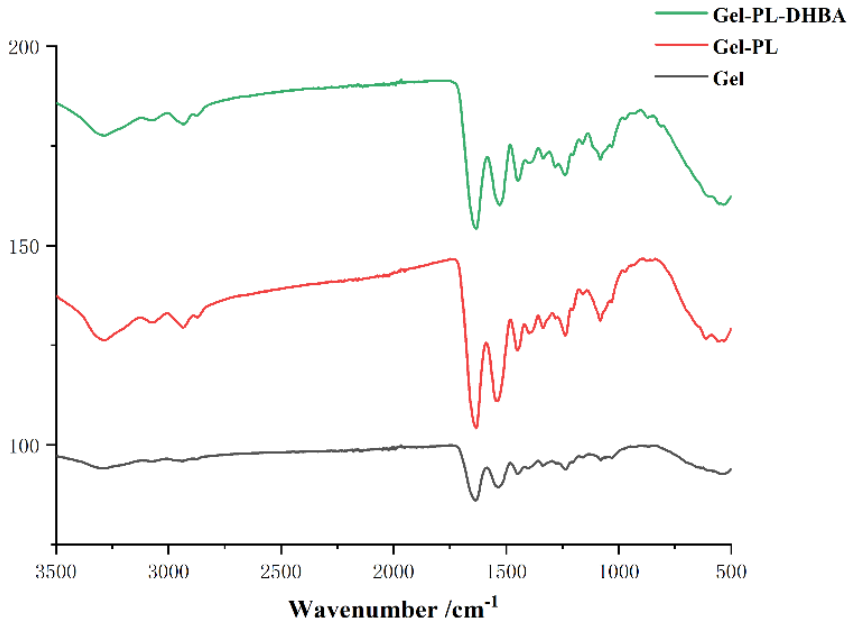


Figure.4 FT-IR of Gel-PL, DHBA and GEL-PL-DB.

As primary analysis to verify the success of graft DHBA onto GEL-PL, ATR-FTIR spectroscopy was adopted in this experiment. As shown in the figure.3, the peaks at 3297 $cm^{-1}$ and 3070 $cm^{-1}$ are from the stretching vibration of N-H, which is due to the presence of primary amino groups in the component. The peak at 2930 $cm^{-1}$ is the characteristic absorption peak of the amide group. The peaks at 1637 $cm^{-1}$ and 1542 $cm^{-1}$ are the characteristic absorption peaks of the amide I band and the amide II band of Gel. The amide I band is caused by the vibration absorption peaks of N-H bond, carbonyl group and C-N, and the amide II band is caused by the bending vibration of N-H bond. The peak at 1279 $cm^{-1}$ can be assigned to catechol in DHBA structure. By comparing Gel-PL and Gel-PL-DB spectra, it can be seen that the addition of DHBA will consume the amino group in Gel-PL to form an amide bond, which is converted into an imine under acidic conditions. This is because the aldehyde group undergoes a Schiff base reaction with the amino group. The amino group acts as an electronegative center and binds to the carbonyl carbon as an electropositive center. Because the electronegativity of oxygen is greater than that of nitrogen, the hydroxyl group is easily removed and dehydrated under acidic conditions. The carbon connected to the hydroxyl group is positively charged, and the lone pair electrons on the nitrogen can bind to the empty orbit of the carbon, so that the hydrogen on the nitrogen becomes proton hydrogen and falls off, thus forming an imine. Therefore, in the Gel-PL-DB reaction system, the number of amino groups decreases, and the content of amide bonds or imines increases. The ratio of the characteristic absorption peak of the N-H bending vibration at 1542 $cm^{-1}$ to the characteristic absorption peak of the C-N and C = N stretching vibration at 1637 $cm^{-1}$ decreases. This shows that DHBA was successfully grafted into the Gel-PL system. This is consistent with the results of Huo et al. [23].

### 3.4 Thermal stability analysis

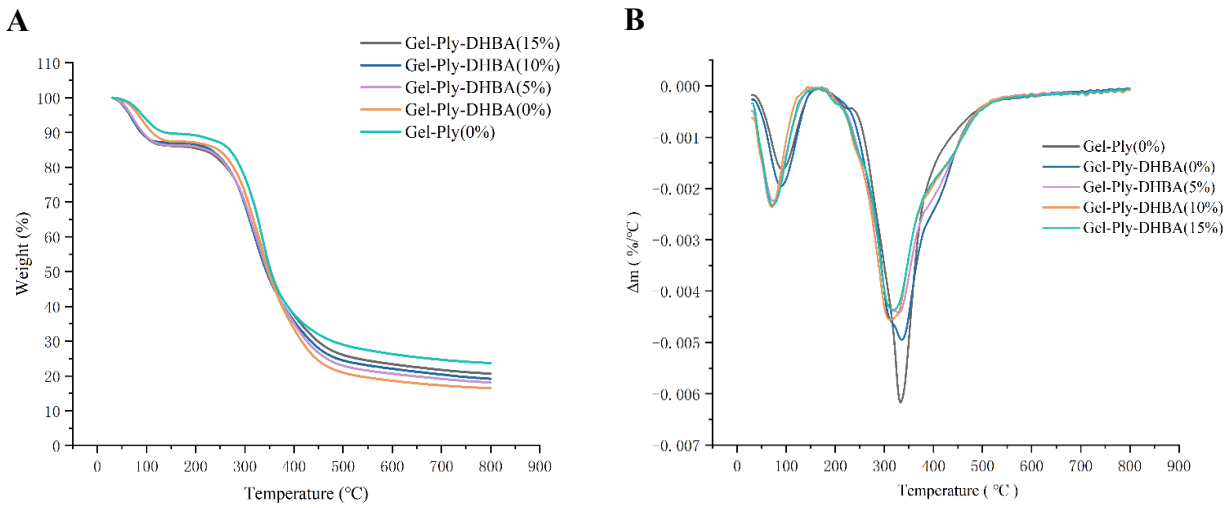


Figure.5 TG(A) and DTG(B) temperature curves of Gel, Gel-PL and Gel-PL-DB adhesives.

Table 1:50% weight loss temperature T50, initial decomposition temperature Td and maximum decomposition rate temperature Tm of hydrogel.

| Samples | $T_{50}$ °C | $T_d$ °C | $T_m$ °C |
|---|---|---|---|
| Gel | 348.17 | 216.55 | 332.50 |
| Gel-PL | 344.08 | 212.42 | 334.67 |
| Gel-PL-DB (5) | 346.50 | 208.32 | 326.83 |
| Gel-PL-DB (10) | 348.16 | 203.67 | 317.17 |
| Gel-PL-DB (15) | 353.17 | 202.35 | 315.33 |

The TG and DTG temperature curves of Gel, Gel-PL and Gel-PL-DB binders are shown in the figure.4. The 50 % weight loss temperature ($T_{50}$), the initial decomposition temperature ($T_d$) and the maximum decomposition rate temperature ($T_m$) of the binder were shown in Table.1. It can be seen that the thermal degradation trend is similar among the as-synthesized Gel-PL-DB as seen from the TGA curves. And all samples show four different weight loss stages in the range of 50-800 °C. The first stage is from 30 ~ 150 °C with a weight loss of 10%, which is the loss of bound water in the samples. The second weight loss stage is between 200 °C and 400 °C, which can be attributed to the degradation of the gelatin chains [24]. The third weight loss stage is 450 to 500 ° C , which is induced the presence of benzene ring [25]. The fourth weight loss stage is above 500 °C, which is assigned to the combustion of the residual material [26]. It can be seen that initial degradation temperatures ($T_d$) and the maximum decomposition rate temperature ($T_m$) of Gel-PL-DB decreases with the increase of DHBA, which can be explained by the fact that the structural stability of Schiff bases is weaker than that of peptide bonds [27].

### 3.5 Adhesion performance analysis

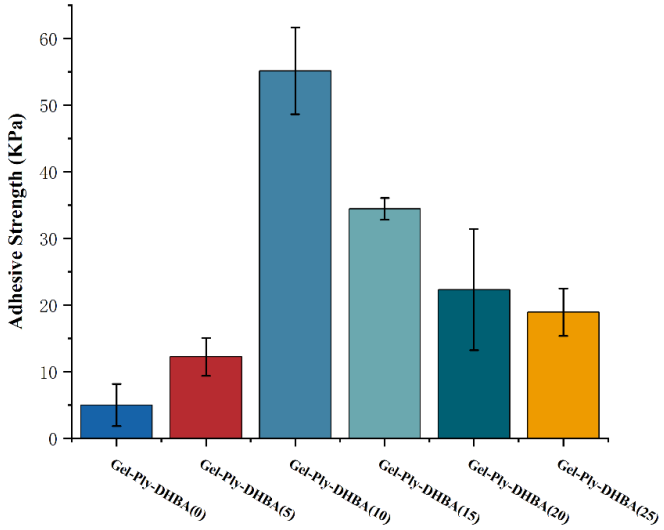


Figure.6 Adhesion strength of Gel-PL-DB.

Adhesion performance is one of the most important properties of adhesives. Strong adhesion performance can ensure that it is not easy to fall off during wound tissue repair, thus playing a role in healing wounds. The adhesive was uniformly coated on the surface of fresh pig skin (fixed area 25 × 25 mm) for lap adhesion experiment (Figure.1). It can be seen from Figure.6 that when the DHBA content is 10 % of the Gel-PL mass, the adhesion strength reaches a maximum of 55.14 ± 6.51 Kpa, which is about 10 times that of Gel-PL (4.98 ± 1.67).

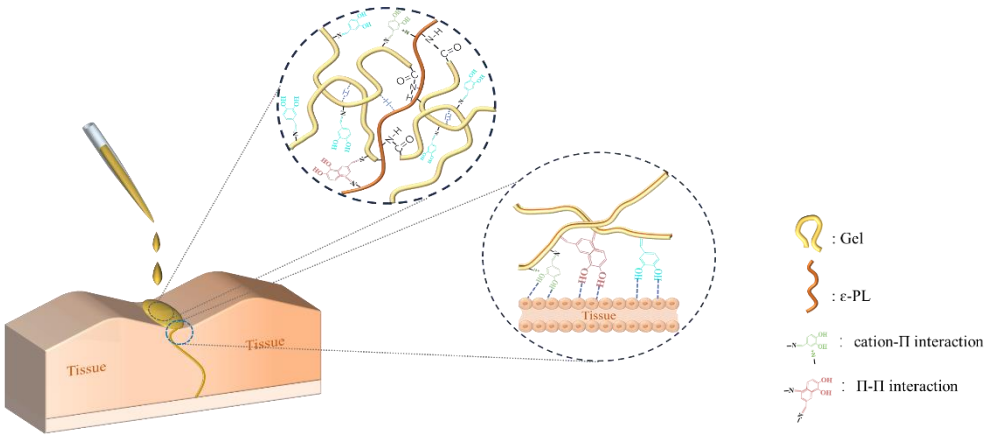


Figure.7 Structure diagram of Gel-PL-DB adhesive and its adhesion mechanism to tissue.

The adhesion mechanism of Gel-PL-DB is schematized in Figure 7. On the one hand, Gel-PL produced a certain covalent cross-linking under the catalysis of transglutaminase. In addition, ε-PL contained a large number of amino groups, showing strong positive charge. The positive charge could interact with the benzene ring in DHBA by cation-π interaction, and there was also a π-π interaction between the benzene rings of DHBA itself, which together enhanced the cohesion of the adhesive. On the other hand, the amino and carboxyl groups contained in the Gel molecule can interact with the amino

and carboxyl groups in the fresh pig skin tissue to produce hydrogen bonding interactions, electrostatic interactions, etc. In addition, the bisphenol structure in the DHBA can also interact with the amino group in the tissue to produce hydrogen bonding interactions. The bisphenol structure is oxidized to a quinone or semi-quinone structure under certain conditions. The structure can react with the amino group in the tissue to produce a certain covalent cross-linking, thereby enhancing the adhesion to the tissue.

## 4 Conclusion

The gelatin-based bio-adhesive, Gel-PL-DB, was synthesized by introduce catechol groups onto ε-PL grafted gelatin with enzymatic crosslinking. The study of FTIR spectra and thermogravimetric analysis provide the graft reaction do takes place. The maximum adhesion strength (55.14 ± 6.51 Kpa) was achieved under the optimum conditions. The bio-adhesive of Gel-PL-DB can be a promising candidate in the biomedical applications.

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