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# Preparation and evaluation of translucent Balsa-Caffeic acid modified lysozyme dressing

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# Preparation and Evaluation of Translucent Balsa-Caffeic Acid Modified Lysozyme Dressing

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**Abstract.** objective: using caffeic acid as modifier, dopamine adhesive prepared modified lysozyme, using bacteriostatic circle diameter, bacteria culture and the minimum bacteriostasis concentration bacteriostatic function, the optimal PH, temperature, time, contrast enzymology properties, and hydrophobicity and secondary structure content determination. Methods: the preparation of translucent Balsa, respectively designed for Control group (Control), wood group (A), translucent Balsa group (B), translucent Balsa - lysozyme group (C), translucent Balsa - Caffeic acid modified lysozyme group (D). Results :(1) the bacteriostatic effect and the minimum inhibitory concentration of the Caffeic acid-modified lysozyme > Caffeic acid; The surface hydrophobicity index of the three substances and the stability of the secondary structure were all caffeic acid - modified lysozyme > lysozyme > Caffeic acid; PH = 6, the temperature is 50°C, the response time of 30 min strongest Caffeic acid modified lysozyme activity; (2) the Drug loading and encapsulation rate of group D were better than that of group C. In terms of the release curve, the release of C and D groups in the 48h group tended to be stable and maximum, and the cumulative release percentage of 72h was 85.2% and 90.5% respectively. (3) in the control group, the growth of staphylococcus aureus and e. coli was basically in line with the normal trend, and the antibacterial activity of e. coli and staphylococcus aureus in each group was D>C>B ($P<0.05$). And 1-7 days, different groups had no value inhibition on fibroblasts ($P>0.05$); this study successfully optimized the design of modified lysozyme to prepare Translucent Balsa-Caffeic Acid Modified, which has strong antibacterial ability, stable and persistent release, and no cytotoxicity.

**Key words:** Balsa; Lysozyme; Caffeic acid; dopamine.



## INTRODUCTION

Lysozyme is a safe means of sterilization protein, but its antibacterial effect is limited to gram-positive bacteria, and the effect on gram-negative bacteria is very small, is a kind of broad spectrum antimicrobial[1-2] .therefore effective preservatives as the actual application is restricted by its limited antibacterial spectrum, greatly limits the promotion of natural enzyme as a preservative, in order to change this limitation, by means of natural enzymes were modified to obtain antibacterial characteristics of gram-negative bacteria is necessary, of lysozyme effective modification methods mainly include physical modification, chemical modification and biological modification method[3-4]. This study using caffeic acid as modifier, dopamine adhesive prepared modified lysozyme, using bacteriostatic circle diameter, bacteria culture and the minimum bacteriostasis concentration bacteriostatic function, the optimal PH, temperature, time, contrast enzymology properties, and hydrophobicity and secondary structure content determination[5].

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# MATERIALS AND METHODS

## Material Preparation

*Preparation of Semi-Transparent Balsa*

Select the Balsa with a density of about 160 kg/m3, perforate with punch, and make a round piece with a diameter of 0.6mm and a thickness of 0.8mm. In the 105±3°C under the environment of dry 24 hours, and then soaked in 1% concentration of NaClO2 - acetate buffer (80°C) PH4.6, 12 h of reaction. After careful cleaning, the sample was removed by using deionized water, followed by ethanol - 1:1 ethanol mixed with acetone - acetone in three steps, 10min each step, and repeated three times. Then infiltration lignin, namely methacrylate (MMA) polymerization formaldehyde solution, heating the oven to 70°C for 4 h. Finally, the infiltrating template is sandwiched between two pieces of glass and is further polymerized with aluminum foil. The polymerization process by heating the oven to 70°C 4 h wood finish.

*Preparation of Caffeic Acid Modified Lysozyme*

It is accurate to say that 60 mg of Caffeic acid is dissolved in 3mL5M NaOH, and the pH of 5M is adjusted to 7.5, and the sterilization deionized water to 8 mL is added, and 160 mg of dopamine is added, and the room temperature is still 1h after completely dissolved. Add 60 mg lysozyme, 30°C constant temperature water bath stirring for 24 h, the reaction after the insoluble part of centrifugal (6000 r/min, 20 min), soluble part on Sephadex G - 25 column, collected with enzyme activity of elution peak, dialysis 1 d under 4°C, enrichment, save after freeze drying.

*Preparation of Semi-Transparent Balsa - Caffeic Acid Modified Lysozyme*

It was said that 131.14mgTris hydrochloric acid was dissolved in 100ml deionized ions, and then the dopaminergic powder was added to 200mg, which was prepared to be 2mg/ml with a PH of 8.5 Tris- dopaminergic solution. Then will Balsa soaking solution with dopamine in 12 h, placed in 37°C table, 100 r/min. Each of them was dissolved in 50ml deionized water by 500mg lysozyme and caffeic acid - modified lysozyme powders. The solution was prepared in 10mg/ml of lysozyme final concentration solution, which was divided into C and D groups respectively. Translucent Balsa with deionized water to clean, the dip into in C, D, and two groups of 12 h in the solution, placed in 37°C table, 100 r/min, out after the reaction at room temperature aseptic saved for later use.



## Optimization and Functional Evaluation of Modified Lysozyme

*Determination of Bacteriostatic Ring*

It was suggested that a certain amount of lysozyme and caffeic acid modified lysozyme were dissolved with 50 mM pH 7.0 phosphate buffer solution, and a sample solution of 20 mg/mL was accurately prepared. The cups and saucers method is adopted to gram positive bacteria (staphylococcus aureus) and gram negative bacteria (e. coli) in vitro bacteriostasis experiment, take 20 mL melting has sterilization of solid cultivation based on petri dishes, let stand after being frozen, take 0.2 mL diluted good concentration for 106 ~ 107 cfu/mL bacteria suspension uniformly coated on the tablet, after 10 min, placed the Oxford cup in a petri dish on the surface, to join different samples of the fluid, cover petri dishes, 37°C constant temperature culture 24 h, bacteriostatic circle diameter of the petri dish. Each sample solution was repeated three times and averaged.

*Bacterial Co-Culture*

Staphylococcus aureus (S.aureus) and Escherichia coli (e. coli) strain amplified bacteria (the bacteria to sleep in the night) to 1*109CFU/ml, and the diluted bacterial solution was 1*104CFU/ml in LB medium, and the OD value was detected by 100ml of the enzyme standard. The OD value was about 0.07, which was the standard. Take two 96 - well plates, snapping the lysozyme and modified enzyme placed in the hole, after each group of three holes, each hole to join 200 ul mixed bacteria liquid, 37°C table 12 h after incubation test each OD value change, repeat three times.

*Determination of Minimum Inhibitory Concentration*

It was suggested that a certain amount of lysozyme and caffeic acid modified lysozyme were dissolved with 50 mM pH 7.0 phosphate buffer solution, and the accurate preparation concentration was 2.00, 1.50, l.00, 0.50 and 0.25 mg/mL. Take 0.2 mL per series of sample liquid in the sterilization test tubes, respectively (0.2 mL concentration of 106 ~ 107 cfu/mL of e. coli and s. aureus, mixing, 37°C constant training 2 h; With sterilization deionized water of different mixture will be diluted 1000 times, 0.2 mL were taken on the tablet has been booing, evenly coated with a coating rod and let stand for a moment, the horse petri dishes, 37°C constant temperature culture 24 h, colony number. The sample solution was replaced with a phosphate buffer of 50 mM pH 7.0. The same operation was used as a control. The growth of bacterial colonies on the tablet was observed, and the concentration of the samples in the tablets with sterile growth was MIC.

## Semi - Transparent Balsa - Modified Lysozyme Function Evaluation

*Drug Loading,Encapsulation Efficiency and Drug Release in Vitro*

Drug loading = actual concentration * volume (ug)/dissolved Balsa - lysozyme or modified enzyme total mass (mg);

Encapsulation efficiency = actual concentration/theoretical concentration.

*Cell Proliferation Inhibition*

To remove the primary fibroblasts from the normal primary generation, the cells can be used when the cells are transferred to the 2nd and 3rd generation. The cell count was prepared by using DMEM medium for the calculation of 2000 cells per hole of 96 orifice plates. Place each material hole, each group of 12 after hole (1 hc-positie measuring four times), each hole to join 150 ul configured medium, 37°C incubator placed, repeated three times.



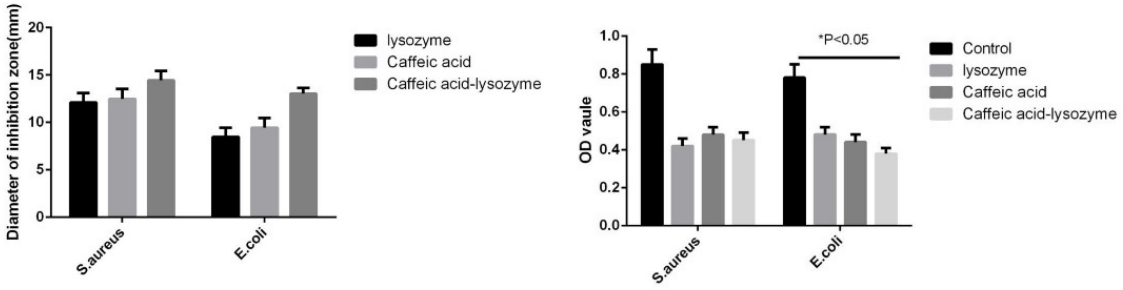
# RESULTS

## Determination of Bacteriostatic Ring, Co-Culture and Minimum Inhibitory Concentration of Bacteria

The bacteriostatic effect and the minimum inhibitory concentration were the Caffeic acid - modified lysozyme > lysozyme > Caffeic acid (figure 1). The surface hydrophobicity index of the three substances and the stability of the secondary structure were all caffeic acid - modified lysozyme > lysozyme > Caffeic acid (figure 2). PH = 6, the temperature is 50°C, the response time of 30 min strongest Caffeic acid modified lysozyme activity (figure 3).

## Drug Loading , Encapsulation Rate and in Vitro Drug Release Research

The Drug loading and encapsulation rate of group D were better than that of group C. From the release curve, the release of C and D groups in the 48h group tended to be stable and maximum, and the cumulative release percentage of 72h was 85.2% and 90.5% (figure 4).




| Strain | Sample | 2mg/ml | 1.5mg/ml | 1mg/ml | 0.5mg/ml | 0.25mg/ml | Control |
|---|---|---|---|---|---|---|---|
| S.aureus | lysozyme | - | - | - | + | ++ | ++ |
| | Caffeic acid | - | - | +- | + | ++ | ++ |
| | Caffeic acid-lysozyme | - | - | - | + | ++ | ++ |
| E.coli | lysozyme | - | +- | + | + | ++ | ++ |
| | Caffeic acid | - | - | +- | + | ++ | ++ |
| | Caffeic acid-lysozyme | - | - | - | +- | + | ++ |

**FIGURE 1.** Comparison of bacteriostatic effect and minimal inhibitory concentration of lysozyme, caffeic acid and modified lysozyme

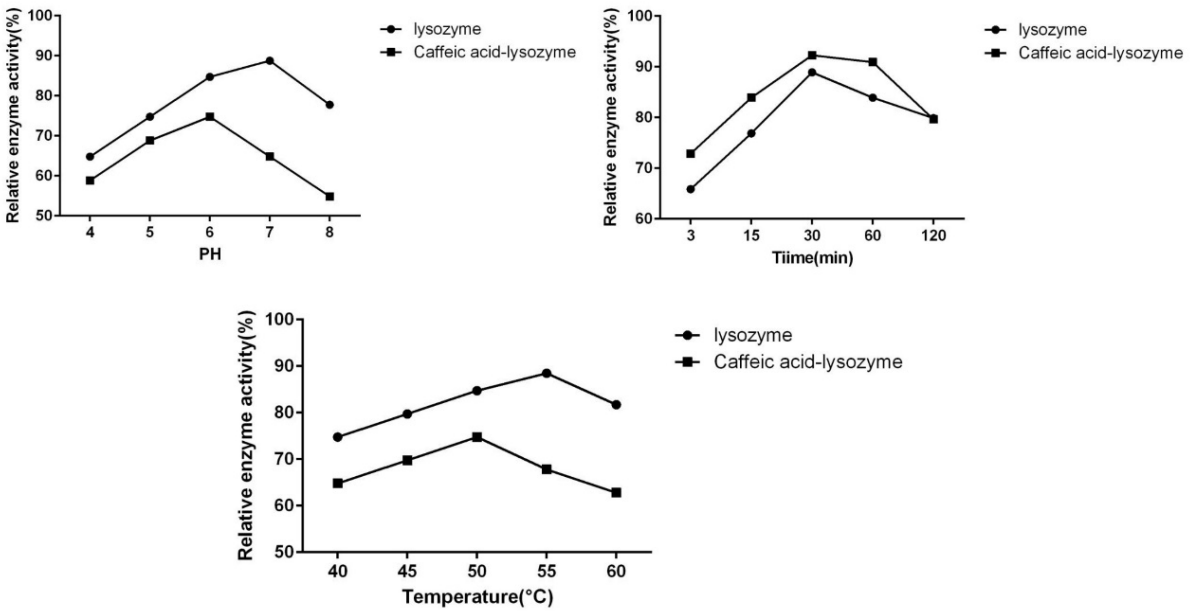
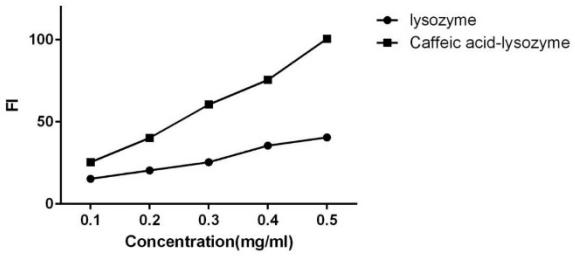



**FIGURE 2.** The optimal temperature, PH and reaction time of lysozyme and modified lysozyme.




| Sample | α-helix | β-sheet | β-turn | Random coil |
|---|---|---|---|---|
| lysozyme | 28.79 | 22.38 | 20.18 | 28.65 |
| Caffeic acid-lysozyme | 24.16 | 20.35 | 21.7 | 33.79 |

**FIGURE 3.** The hydrolysis of lysozyme and modified lysozyme and the comparison of secondary structure.

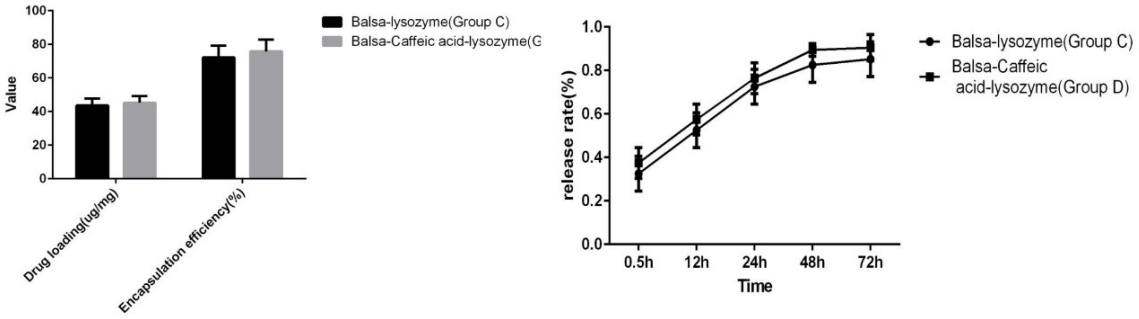



**FIGURE 4.** Drug loading, encapsulation rate and in vitro drug release study of different materials.

## In Vitro Antibacterial and Cell Proliferation Inhibition

In the control group, the growth of staphylococcus aureus and e. coli was basically consistent with the normal trend, and the antibacterial activity of e. coli and staphylococcus aureus in each group was D>C>B (P<0.05). And 1-7 days, different groups had no value inhibition on fibroblasts (P>0.05) (figure 5).

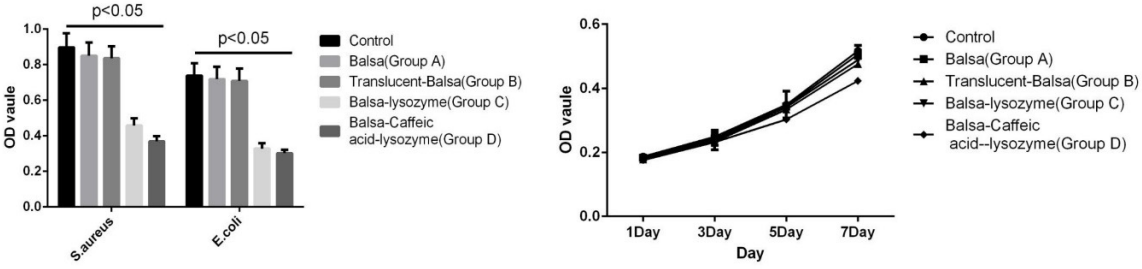



**FIGURE 5.** Comparison of antibacterial and cell proliferation inhibition in vitro in different materials

## DISCUSSION

As shown in figure 1, bacteriostatic effects, and the minimum bacteriostasis concentration for caffeic acid - modified lysozyme > lysozyme > Caffeic acid, showed the modified enzymes to negative bacteria inhibition effect is

greater than the natural enzyme, widening the antibacterial spectrum of natural enzymes [6].PH = 6, the temperature is 50°C, the response time of 30 min strongest Caffeic acid modified lysozyme activity (figure 2). We believe that after chemical modification of lysozyme, the optimum reaction pH change, may be because the natural enzyme, is a combination of different acid, isoelectric point change, caused by the structure of the enzyme also changes[7-8]. Three kinds of enzyme surface hydrophobicity of the index of caffeic acid modified lysozyme > lysozyme > caffeic acid (figure 3), probably because after the modification of lysozyme, some changes have taken place in structure, which was wrapped in natural enzyme exposed groups within the hydrophobic molecules, increased the surface hydrophobic modification enzyme index[9-10].

In this study, the drug loading and encapsulation rate of group D were better than that of group C ($P<0.05$). From the release curve, the release of C and D groups in the 48h group tended to be stable and the maximum value was 85.2% and 90.5% respectively (figure 4). Reason may be that in the process of preparation of microspheres, caffeic acid modified lysozyme and may are more likely to be coated on the surface Balsa, and pure lysozyme due to excessive concentrations less actual glue of couplet of lysozyme content, makes the envelopment rate reduced. In the control group, the growth of staphylococcus aureus and e. coli was basically consistent with the normal trend, and the antibacterial activity of e. coli and staphylococcus aureus in each group was D>C>B ($P<0.05$) (figure 5). According to relevant data the exposure of the enzyme molecule hydrophobic groups is the main reason for the enhanced of gram-negative bacteria[11-12], modification of enzyme of hydrophobic index increase further for its effect on gram-negative bacteria increase provides theory basis. In the case of cytotoxicity, there was no increased inhibition of fibroblasts in different groups ($P>0.05$) (figure 6). This may be related to lysozyme from egg white, a natural, safe enzyme. It is believed that the safety of lysozyme makes it widely used in food preservation [13].

To sum up, this study successfully optimized the design of modified lysozyme, prepared semi-transparent and Balsa - modified lysozyme dressing, which has strong antibacterial ability, stable and persistent release, and no cytotoxicity.

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