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Explore the potential application of glycyrrhizic acid ammonium salt strontium pectin(GasSP) and its components(Gas and SP) in the medical field

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Abstract:

Objective: Finding the potential application of glycyrrhizic acid ammonium salt strontium pectin (GasSP) and its components (Gas and SP) in the pharmaceutical field. Methodology: Searching and summarizing literature on strontium, pectin, and Gas; designing and summarizing expert interview to figure out potential applications of GasSP and its components; designing and manipulating experiments to figure out the antibacterial properties of GasSP with 5 different concentrations and Gas with 6 different concentrations. Results: No significance difference in the antibacterial properties were found between each group of GasSP; All of the treatment groups of GasSP were observed to be not antibacterial; And there was a positive correlation between the Gas concentration and the antibacterial properties of Gas. Conclusions: This study provides a description of antibacterial properties and potential applications of GasSP and its components. The research showed that GasSP can be used as a skin wound dressing, which serves a groundbreaking basis for the future studies in medical field.

Keywords: GasSP; Glycyrrhizic acid ammonium salt strontium pectin; Glycyrrhizic acid ammonium salt; Strontium; Pectin; Pectin-based hydrogel; Wound dressing; Antibacterial property.

1. Introduction

In recent years, there has been an increasing interest in polymer hydrogel which is generally understood to mean a new three dimentional structure. according to Zhang Qian, the polymer hydrogel is extensively used in medical field because of its good biodegradability and biocompatibility, similar structure to biological tissue, and numerous of active group in the polymer chain. many researchers have worked on the applications of a variety of hydrogel, for instance, polymer hydrogels are used in scaffold for tissue engineering, wound dressing, drug delivery system, but there are few studies about glycyrrhizic acid

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ammonium salt strontium pectin (GasSP)-an ionic cross-linked low-fat pectin hydrogel with glycyrrhizic acid ammonium salt and strontium. according to the preliminary experiment, the GasSP can promote the growth of specific bacteria to a certain extent, therefore, it is important to explore the potential applications of the bacterial growth promoting GasSP in order to fill the research gap from the dual-theoretical and empirical research perspective.

In this paper, the antibacterial properties of glycyrrhizate strontium pectin (GasSP), glycyrrhizic acid ammonium salt (Gas), strontium pectin(SP) are respectively organized in a targeted manner, and the potential applications of these three substances in the field of food and drug are summarized. it is expected that this thesis could provide a reference basis for realizing the further development and utilization of strontium pectin hydrogels and glycyrrhizic acid ammonium salt.

2. Literature References

The preparation and application of hydrogels have become a more prominent area of research in recent years. The properties of hydrogels can be varied according to its different components, preperation methods and so on.

This study is dedicated to explore the application potential of GasSP and its components, the existing studies on each of the three components are summarised below.

2.1 Studies on Glycyrrhizic Acid Ammonium Salt

Glycyrrhizic acid(GA) is the major sweet-tasting constituent of Glycyrrhiza glabra root which is a herb that grows perennially in central and south-western Asia and the Mediterranean region. The GA molecule consist of two parts: hydrophobic part with glycyrrhetinic acid, and hydrophilic part with two glucuronic acid molecules[1]. Moreover, it is hydrolysed by commensal bacteria in the intestine into glycyrrhetinic acid (enoxolone; glycyrrhetic acid) which is a pentacyclic triterpenoid derivative of the beta-amyrin type[2]. A comparative study concluded that the pharmacological effects of GA are essentially those obtained form glycyrrhetinic acid[1].

Multiple therapeutic areas have been shown to benefit from GA, including anti-inflammatory, anti-ulcer, anti-allergic, antioxidant, anti-tumor, anti-diabetes, and hepatoprotective properties[1, 3]. Anti-patrasic properties were also demonstrated by GA[4, 5]. What's more, GA has a great activity in improving the mentioned metabolic conditions, especially in improving the insulin sensitivity and lipid profile[3]. The derivatives of GA was demonstrated to have a wide range of pharmacological properties, including anti-inflammatory, antidote, anti-ulcer, antiviral, anti-allergic and some other properties[6]. It had also been demonstrated that there was a strong antibacterial effect of glycyrrhetinic acid and its derivatives (includes ammonium salt) on specific strains of S. aureus [7]

2.2 Studies on Strontium

Strontium is a silver-white or yellowish alkaline earth metal which behaves similarly to the other alkaline earth metals in group 2[8]. According to the statisics, the global production of strontium in 2020 was 210,000 tonnes. Due to its ability to chemically react with air and water, strontium do not naturally has a free state and is instead a form of compounds[9] (mostly in the form of celestite, $SrSO_4$ and strontianite, $SrCO_3$). Strontium exist as four stable types of isotopic forms, each of which can be written as 84Sr, 86Sr, 87Sr, and 88Sr, and they are all non-radioactive[10]. Strontium can also be present in various radioactive isotopes, with 90Sr being the most prevalent. The formation of Strontium-90 occurs either in nuclear reactors or during the explosion of nuclear weapons. 90Sr generates beta particles and has a half-life of 29 years[10]. Up to now, data from several sources have identified the radioactive isotope 89Sr and 90Sr is used as a cancer therapeutic to damage the tumor cell in vivo by eliminating beta radiation[11]. A study of Bin Niu in Asian Journal of Surgery offers probably the most practicle evidence that strontium-90 successfully reduced the size of subungual glomus tumor for 3 times[12]. The study by A. T. Porter reported that metastron (Strontium-89 Chloride injection[13]) can significantly reduce the local pain in patients with prostate cancer metastatic to the bone[11]. Other studies indicated that strontium is able to extends the lifespan of osteoblasts, and promote recruitment of endothelial cells and formation of blood vessels[14]. At the same time, strontium is able to reduce the differentiation of macrophages into osteoclasts by inhibiting the expression of specific factors, reducing the number of osteoclasts and thus promoting new bone formation[15].

2.3 Studies on Pectin & Pectin Based Hydrogel

In the researches of hydrogel, hydrogel based on ion complex is defined as an indispensable area that is popular among the researchers. Pectin, as a multifunctional plant polysaccharide present in the plant cell wall[16], has excellent properties such as hemostatic, detoxification, pro-osteoblastic[17] and hypolipidemic[18], which leads to its potential for a wide range of applications in the pharmaceutical and food industries. Most pectin-based hydrogels' preparations utilize the coordination of low methoxyl pectin (LMP) with cations such as calcium. However, the molecular structure of pectin largely limits its strength, toughness, and stability of the hydrogels. Much of the current literature on pectin-based hydrogel pays particular attention to the methods for improving the

stability of hydrogels for use them in applications such as hydrogel dressings, prodrug, drug or living bacteria encapsulation, and so on. In a study which set out to determine the properties of pectin derivative with quaternary ammonium salts (QP) which were prepared by reacting pectin with 3-Chloro-2-hydroxypropyltrimethyl ammonium chloride, Wang Tan found that the in vitro cytotoxicity test of the QP showed no significant cytotoxicity, the in vitro antimicrobial test showed that the QP exhibited significant antibacterial effects against Staphylococcus aureus, Staphylococcus epidermidis, Bacillus, Candida albicans, Candida parapsilosis and Malassezia[19]. This view is also supported by Lei Wanxue who demonstrated that the prepared QP have a good bactericidal effects on Staphylococcus aureus, Escherichia coli, and Candida albicans, with a 99.99% bactericidal rate after contact for 30 minutes[20]. The first serious discussions and analyses of the term "prodrug" emerged during the 1958s with the prodrug itself has no biological activity or very low activity, and is transformed into an active drug (compound with pharmacological effects) only after it has been converted in the organism by a chemical reaction or by enzymes, this process aimed at increasing the bioavailability of the drug, enhancing targeting, and reducing the toxicity and side effects of the drug[21]. Recent evidence suggests that pectin is specifically responsive to the enzymes/flora in the colon, making it suitable for use as a carrier for orally colon-targeted prodrugs, this type of approach is only applicable to drugs with functional groups that can be coupled to pectin[22]. It is also proved that pectin exhibits prebiotic activity and stimulates in situ growth of probiotics. Low methoxy pectin (LMP) can react with cations (e.g. calcium) to form gels for encapsulation of substances including probiotics, antioxidants, enzymes, or substances with other bioactivities, enabling colon-targeted drug delivery systems in the same literature.

2.4 Summary

According to previous literature, Gas is significantly antimicrobial. While strontium has the effect of promoting osteoblasts and angiogenesis. What's more, pectin derivatives have indeed been shown to have strong antibacterial effects, but pectin itself has the effect of detoxifying and improving bioactivity. Overall, there are relatively few antimicrobial analyses of strontium and pectin.

3. Methodology

3.1 Overview

To validate the results of previous studies on the antibacterial properties of Gas, and explore the antibacterial properties of GasSP as a newly developed substance. This study investigated the antibacterial properties of GasSP and Gas in different concentrations by photoelectric turbidity test and zone of inhibition test. So that these results could fill the gap in the study of the antibacterial properties of GasSP and its components.

3.2 Research Topic & Research Method

The primary aim of this paper is to provide empirical and theoretical evidence for the possible application of GasSP and its components, in order to evaluate the feasibility of each application of these substances, the type of this research is interpretivism, it is determined to use quantitative researches to acquire the antibacterial properties of Gas and SP against S.aureus, P.aeruginosa and escherichia coli by conducting phtoelectric turbidity experiment and zone of inhibition test, and demonstrate the other properties and potential applications of Gas and SP by secondary researches and a semi-structurd interview with an expert in the field of biomaterial. The advantages of combining quantitative and qualitative method is that this enhances the study's professionalism and completeness. Because we can obtain social data from this discussion, as well as professional data from statistics and analysis.

3.3 Procedure of Two Sets of Photoelectric Turbidity Experiments of GasSP

3.3.1 Materials & Devices

n Lyophilized GasSP with different Gas concentration--0%, 0.5%, 1%, 2% (w/v during GasSP preperation)
n Lysogeny broth (LB)
n S.aureus, P.aeruginosa and escherichia coli culture fluid
n Swiss TECAN multifunctional enzyme marker Spark
n Clean bench
n Other laboratory consumables

3.3.2 Pre-preparation of Lyophilized GasSP

The preparation of GasSP utilize the coordination of low methoxyl pectin (LMP) with cations calcium to form pectin hydrogel, then the strontium element is added using ionic crosslinking, finally, add Gas into the hydrogel using ionic crosslinking and conduct lyophilization to form the lyophilized GasSP.

3.3.3 Pre-preparation of lysogeny broth (LB)

Add the following components into 950ml de-liquefied LB medium ionized water: Casein Tryptone 10g, yeast extract 5g, NaCl 10g and shake the vessel until the solutes are dissolved. Adjust the pH to 7.0 with 5 mol/L NaOH. Bring to volume by deionised water to 1 L. Steam sterilise at 15psi under high pressure for 20 min.

3.3.4 Operating Steps

Step 1:

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The initial reagents of the experimental group and the control group were set in two sets of experiments for each type of bacteria.

Table 1. Initial Reagent in First Set of Experiments

Experiment 1		
	LB(ml)	GasSP with different w/v of Gas(mg)
Control group A	5	0
Treatment group A	5	1 (0% w/v)
Treatment group C	5	1 (0.5% w/v)
Treatment group E	5	1 (1% w/v)
Treatment group G	5	1 (2% w/v)

Table 2. Initial Reagent in Second Set of Experiment

Experiment 2		
	LB(ml)	GasSP with different w/v of Gas(mg)
Control group B	5	0
Treatment group B	5	9 (0% w/v)
Treatment group D	5	9 (0.5% w/v)
Treatment group F	5	9 (1% w/v)
Treatment group H	5	9 (2% w/v)

Step 2:
Pending the release of the drug for a specific length of time, extract supernatant fluid.
Leave the control group A,B reagents at -20℃. Reagents of treatment group A,B,C,D,E,F,G are then oscillated until GasSP is fully dissolved in the LB solution. After that, the initial reagents of the experimental group A,C,E,G were placed for 24 hours and the initial reagents of the experimental group B,D,F,H for 96 hours. Centrifuged the reagents of the experimental group A,B,C,D,E,F,G under a condition of 3000rpm, 5 minutes and 23℃, then, extract 500μL of supernatant fluid in each reagent.
Step 3:
Add 50μL of S.aureus/ P.aeruginosa/ escherichia coli culture fluid into 500μL of supernatant fluid of each reagent, ensure that the mixture are fully blended evenly.
Step 4:
Add each group of mixture to three holes seperately in the 96-well cell culture plate (3 times parallel repetition), 100μL of mixture per hole.
Step 5:
Put the 96-well cell culture plate into the ELISA Analyzer, test the OD600 value.
Step 6:
Draw a line graph to represent the relationship between GasSP with different concentrations of Gas and OD values for each bacterium.

3.4 Procedure of Zone of Inhibition Test of GasSP

3.4.1 Materials & Devices

n GasSP without lyophilization with different Gas concentration--0%, 0.5%, 1%, 2% (w/v during GasSP preperation)
n S.aureus, P.aeruginosa and Escherichia coli culture fluid
n Solid lysogeny broth(LB) medium
n Clean bench
n Coater
n Alcohol burner
n Other laboratory consumables

3.4.2 Pre-preparation of Solid Lysogeny Broth(LB) Medium

Add the following components into 950ml de-liquefied LB medium ionized water: Casein Tryptone 10g, yeast extract 5g, NaCl 10g and shake the vessel until the solutes are dissolved. Add 15g agar powder, antibiotics before the temperature drops to 40 ° C, and import into the dish. Adjust the pH to 7.0 with 5 mol/L NaOH. Steam sterilise at 15psi under high pressure for 20 min.

3.4.3 Operating Steps

Step 1:
The initial materials of the experimental group and the

control group were set for each type of bacteria.

Table 3. Initial Material

Experiment 3	
	GasSP without lyophilization with different w/v of Gas
Control group X	1 piece with constant size(0% w/v)
Treatment group Y	1 piece with constant size(0.5% w/v)
Treatment group Z	1 piece with constant size(1% w/v)
Treatment group M	1 piece with constant size(2% w/v)

Step 2:
Took a small amount of S.aureus/ P.aeruginosa/ escherichia coli culture fluid (no more than 200μL) and drop it evenly to the surface of the medium;
Step 3:
Aseptic coater were applied uniformly to the surface of the medium.
Step 4:
Dispersedly distributed group X,Y,Z,M onto the petri dish surface and wait for 12h to observe the zone of inhibition.

3.5 Procedure of Photoelectric Turbidity Experiment of Gas

3.5.1 Materials & Devices

n Glycyrrhizic acid ammonium salt(Gas)
n Lysogeny broth (LB)
n S.aureus, P.aeruginosa and escherichia coli culture fluid
n Swiss TECAN multifunctional enzyme marker Spark
n Clean bench
n Other laboratory consumables

3.5.2 Pre-preparation of Lysogeny Broth(LB)

Add the following components into 950ml de-liquefied LB medium ionized water: Casein Tryptone 10g, yeast extract 5g, NaCl 10g and shake the vessel until the solutes are dissolved. Adjust the pH to 7.0 with 5 mol/L NaOH. Bring to volume by deionised water to 1 L. Steam sterilise at 15psi under high pressure for 20 min.

3.5.3 Operating Steps

Step 1:
The initial reagents of the experimental group and the control group of experiments for each type of bacteria.

Table 4. Initial Reagent

Experiment 4		
	LB(ml)	Gas(mg)
Control group C	3	0
Treatment group I	3	10
Treatment group J	3	20
Treatment group K	3	30
Treatment group L	3	40
Treatment group M	3	50

Step 2:
Extracted the Gas reagents with no impurities.
Leave control group C reagents at -20℃. Reagents of treatment groups I, J, K, L, M are then oscillated until the Gas is fully dissolved in the LB solution. Then, use syringe filters to extract 500μL of fluid from each reagents of group C, I, J, K, L, M.
Step 3:
Add 50μL of S.aureus/ P.aeruginosa/ escherichia coli culture fluid into 500μL of each reagent, ensure that the mixture are fully blended evenly.
Step 4:
Add each group of mixture to three holes separately in the 96-well cell culture plate (3 times parallel repetition), 100μL of mixture per hole.
Step 5:
Put the 96-well cell culture plate into the ELISA Analyzer, test the OD600 value.
Step 6:
Draw a line graph to represent the relationship between Gas with different concentrations and OD values for each bacterium.

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3.6 procedure of semi-structured interview

Apart from carrying out experiments to figure out the antibacterial properties or GasSP and Gas. This study also conduct a semi-structured expert interview in order to have a better understanding on the application of GasSP.

To ensure the privacy and confidentiality of the data of the interviewees, the interviewees signed a written consent form and agreed to cooperate with the interviewees.

The semi-structured approach was chosen because a pre-determined list of topics was available prior to the official interview, which allow the interviewee to get a preliminary idea of the topics in advance, making the answers in the official interview more logical and referential. In addition, the order in which the questions were discussed and the increase or decrease in content were relatively flexible and depend on what the interviewee say.

The interviewee of this study is a tenured professor from Southern University of Science and Technology, who has been farming in the field of biomaterials for many years and has a high academic reputation.

This interview was conducted as an online conference, and to ensure the effectiveness of the answer, the interviewer created a comfortable and supportive atmosphere throughout the process, encouraged the interviewee to respond honestly and open-minded. In addition, the answers given by the interviewee in an unfamiliar environment are likely to deviate from those given in a natural state based on the reactive effect, so the interviewee was encouraged to conduct interview in familiar environment to maximize the effectiveness of the study.

4. Result

4.1 Experiment 1

4.1.1 Overview

This experiment aimed to test the antibacterial property of GasSP with different Gas concentrations against P.aeruginosa, Escherichia coli and S.aureus. (24h of drug releasing time; 1mg of Gas inside GasSP)

4.1.2 Summary

In the first set of experiment, there was no significant effect of different concentration of Gas inside the GasSP on the growth curve of any type of bacteria. At the same time, none of the differences between treatment groups and control group were statistically significant. Further statistical analysis showed that the standard error of mean (Mean±SEM) between each group was considerably small.

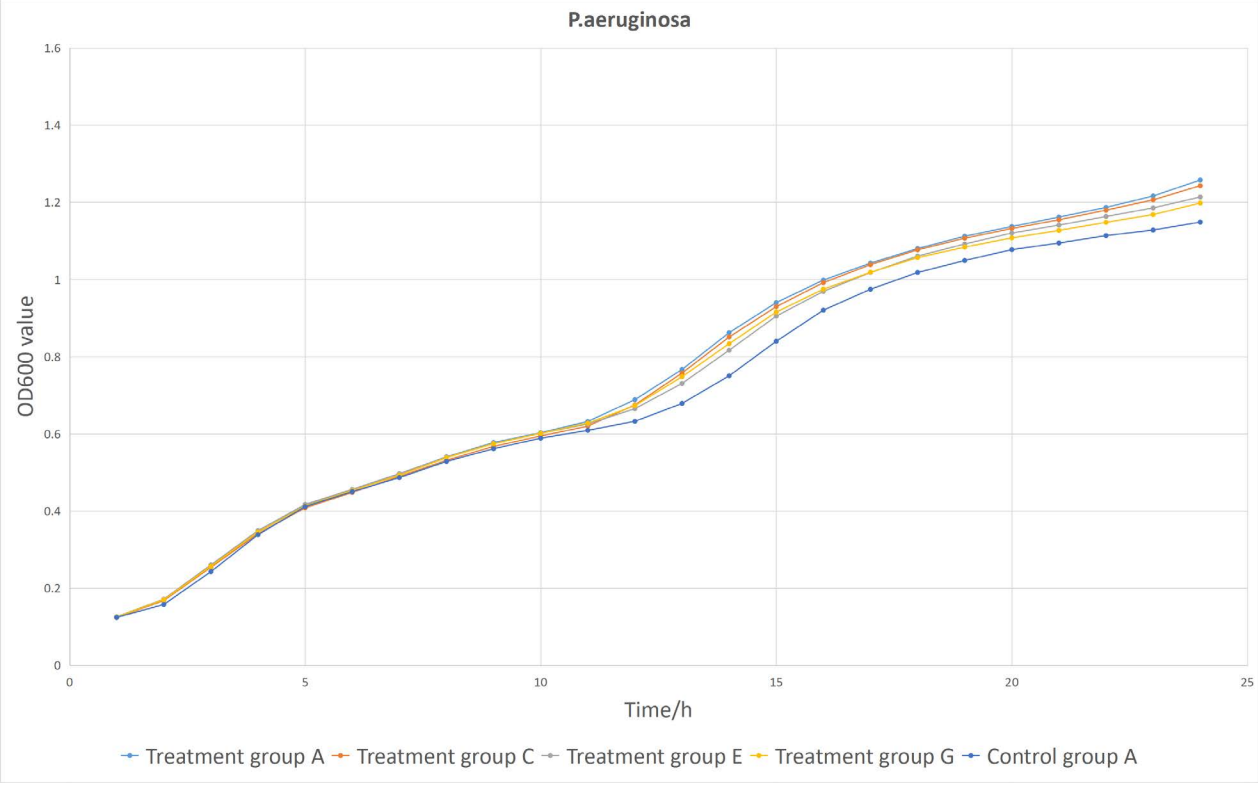

Figure 1. Bacterial growth curve of P.aeruginosa

(Bacterial growth curve of P.aeruginosa for 24h growth in the mixture of lysogeny broth and GasSP with different

w/v of Gas. The control was 0mg of GasSP, the treatment was 1mg of GasSP with 0%, 0.5%, 1%, 2% w/v Gas inside.)

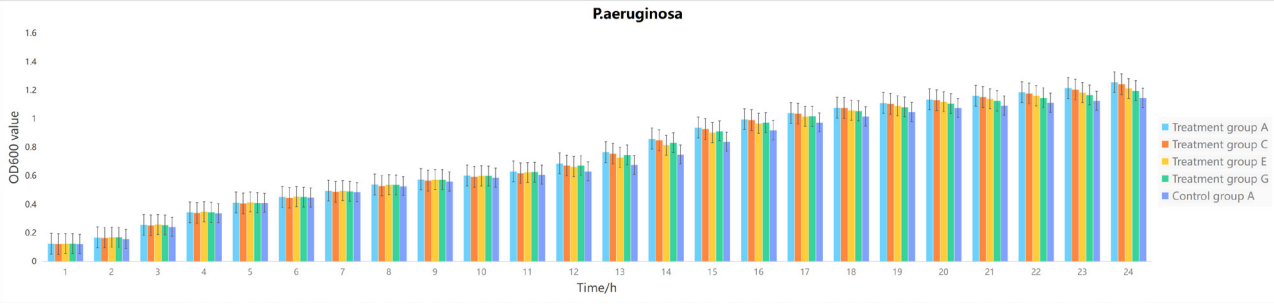

Figure 2. Standard error of mean (Mean±SEM) of OD values of P.aeruginosa per hour between each groups

(Standard error of mean (Mean±SEM) of OD values of P.aeruginosa per hour between each groups during 24h growth in the mixture of lysogeny broth and GasSP with different w/v of Gas. The control was 0mg of GasSP, the treatment was 1mg of GasSP with 0%, 0.5%, 1%, 2% w/v Gas inside.)

For P.aeruginosa (figure 1 and 2), following the addition of Gas concentration among the treatment groups, a marginal increase in the antibacterial property of GasSP was recorded. Surprisingly, from the chart, it can be seen that the treatment groups are moderately more bacterial growth promoting compare to the control group.

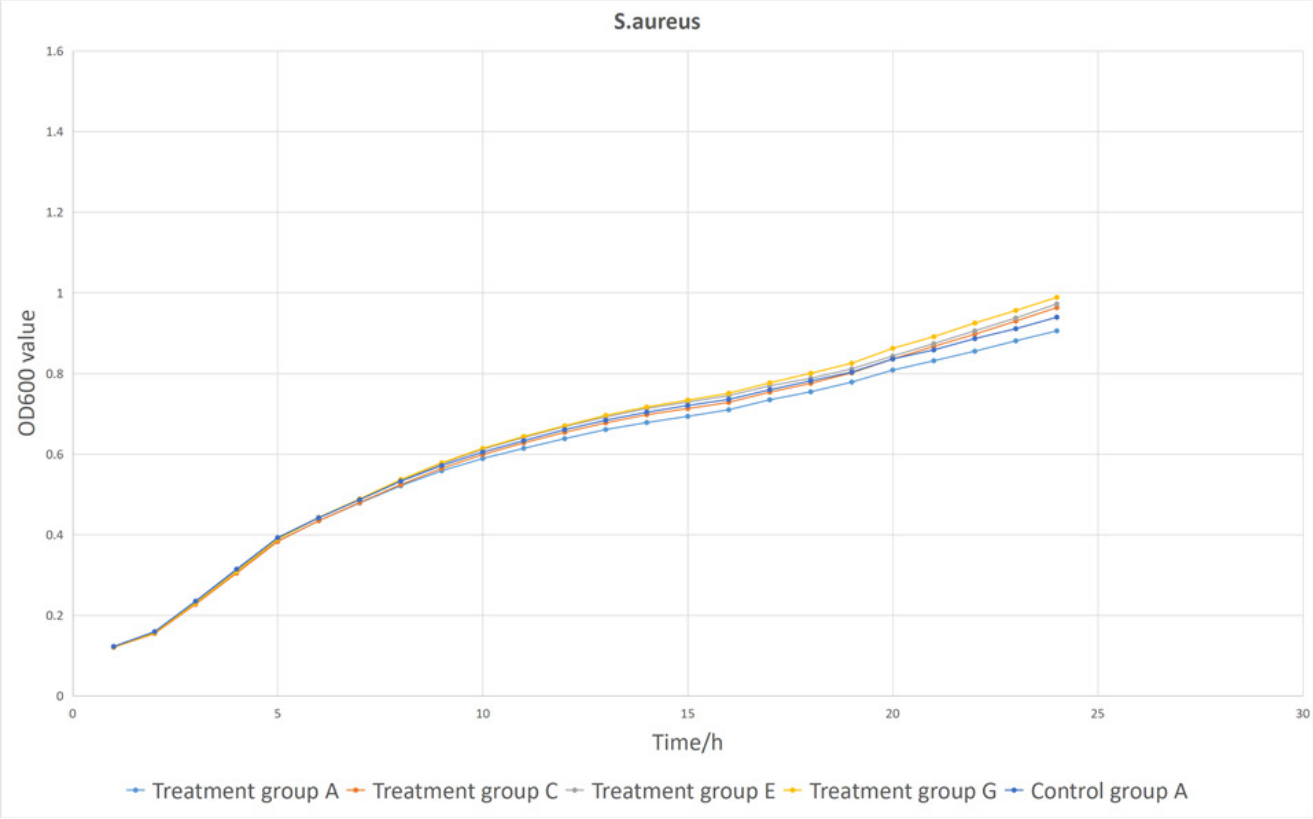

Figure 3. Bacterial growth curve of S.aureus

(Bacterial growth curve of S.aureus for 24h growth in the mixture of lysogeny broth and GasSP with different w/v of Gas. The control was 0mg of GasSP, the treatment was 1mg of GasSP with 0%, 0.5%, 1%, 2% w/v Gas inside.)

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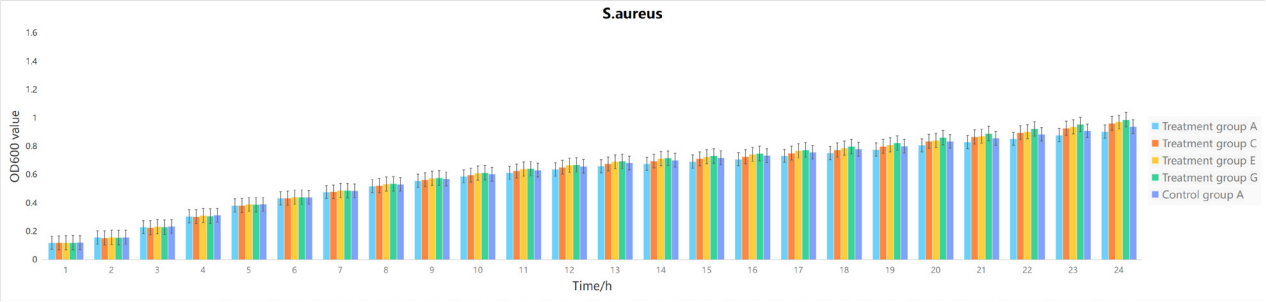

Figure 4. Standard error of mean (Mean±SEM) of OD values of S.aureus per hour between each groups

(Standard error of mean (Mean±SEM) of OD values of S.aureus per hour between each groups during 24h growth in the mixture of lysogeny broth and GasSP with different w/v of Gas. The control was 0mg of GasSP, the treatment was 1mg of GasSP with 0%, 0.5%, 1%, 2% w/v Gas inside.)

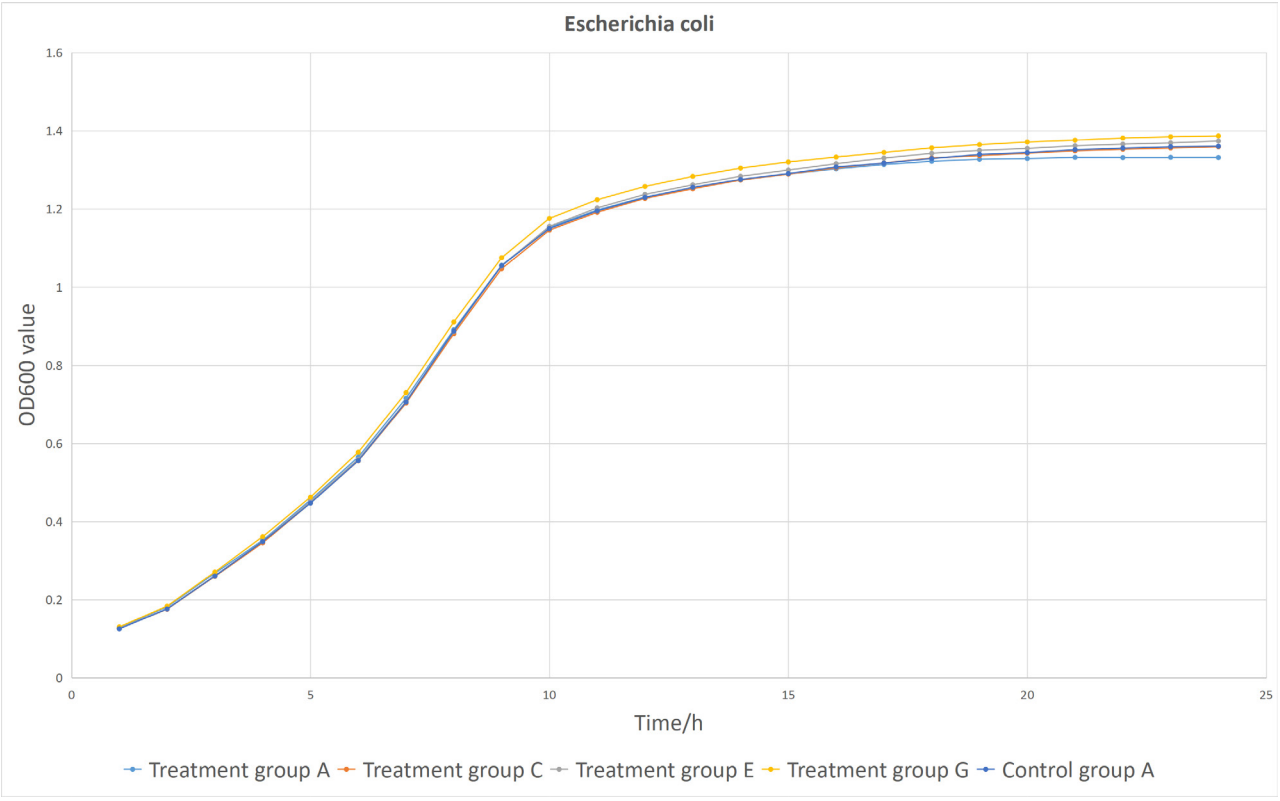

Figure 5. Bacterial growth curve of Escherichia coli

(Bacterial growth curve of Escherichia coli for 24h growth in the mixture of lysogeny broth and GasSP with different w/v of Gas. The control was 0mg of GasSP, the treatment was 1mg of GasSP with 0%, 0.5%, 1%, 2% w/v Gas inside.)

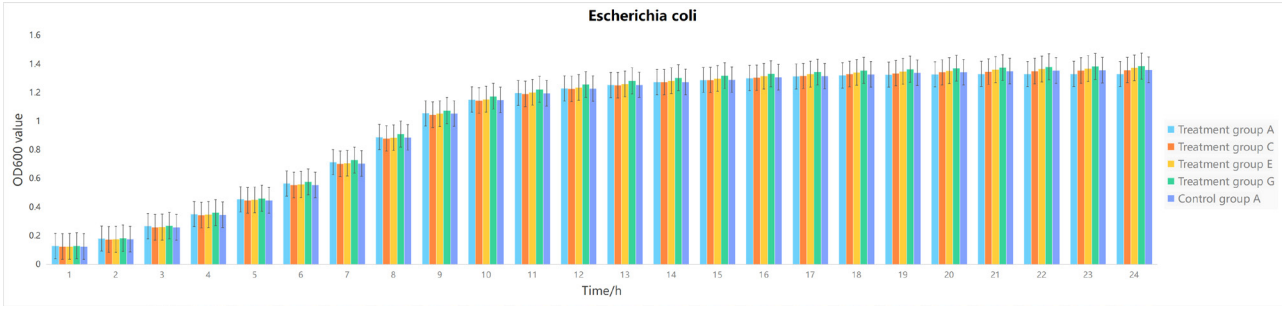

Figure 6. Standard error of mean (Mean±SEM) of OD values of Escherichia coli per hour between each groups

(Standard error of mean (Mean±SEM) of OD values of Escherichia coli per hour between each groups during 24h growth in the mixture of lysogeny broth and GasSP with different w/v of Gas. The control was 0mg of GasSP, the treatment was 1mg of GasSP with 0%, 0.5%, 1%, 2% w/v Gas inside.)

For S.aureus and Escherichia coli (figure 3, 4, 5 and 6), following the addition of Gas concentration among the treatment groups, a marginal decrease in the antibacterial property of GasSP was observed, this result is somewhat counterintuitive. In addition, no significant differences were found between each group, and the antibacterial properties of each group had no direct correlation with the variables in this experiment.

4.2 Experiment 2

4.2.1 Overview

This experiment aimed to test the antibacterial property of GasSP with different Gas concentrations against P.aeruginosa, Escherichia coli and S.aureus.(96h of drug releasing time; 9mg of Gas inside GasSP)

4.2.2 Summary

An increase in the significance difference between treatment groups and control group due to the increase in drug releasing time and concentration of GasSP in the solution was observed in the charts.

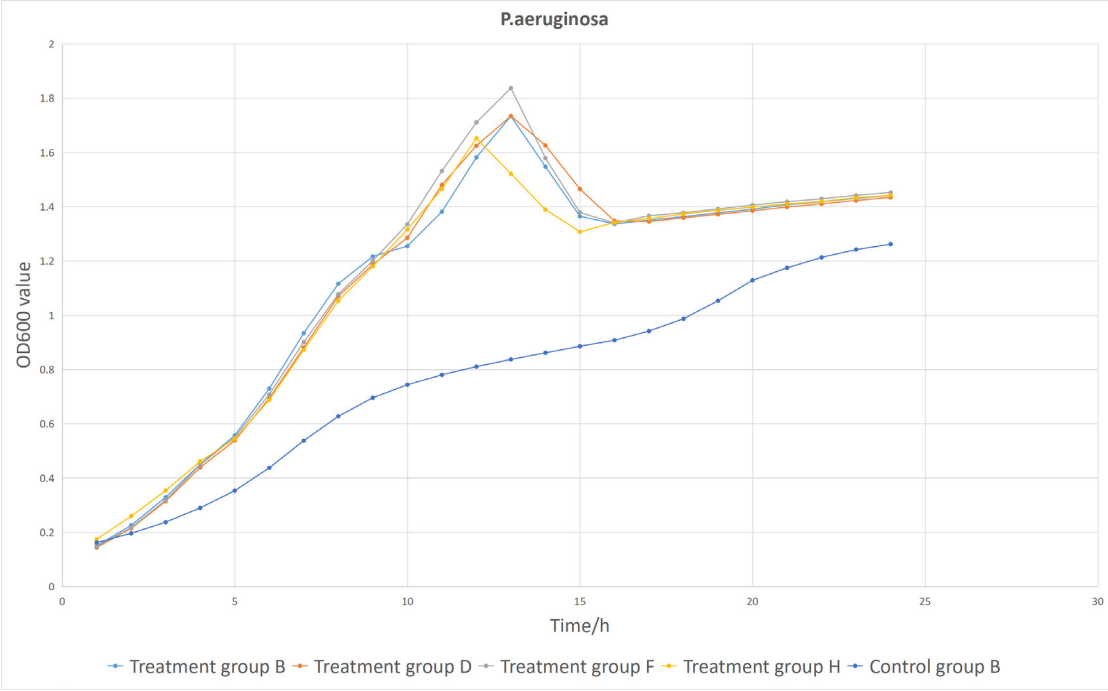

Figure 7. Bacterial growth curve of P.aeruginosa

(Bacterial growth curve of P.aeruginosa for 24h growth in the mixture of lysogeny broth and GasSP with different w/v of Gas. The control was 0mg of GasSP, the treatment was 9mg of GasSP with 0%, 0.5%, 1%, 2% w/v Gas inside.)

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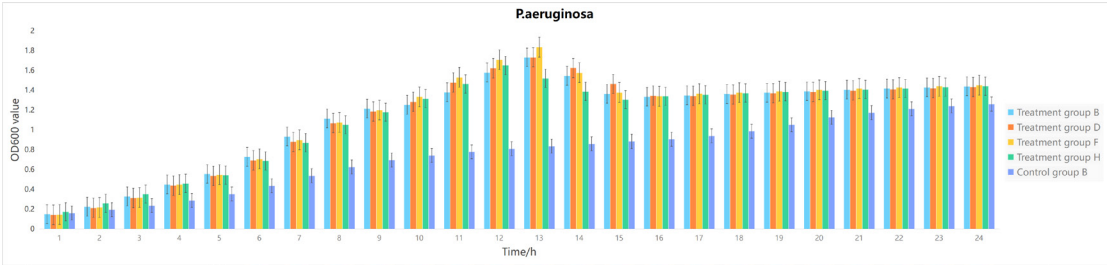

Figure 8. Standard error of mean (Mean±SEM) of OD values of P.aeruginosa per hour between each groups

(Standard error of mean (Mean±SEM) of OD values of P.aeruginosa per hour between each groups during 24h growth in the mixture of lysogeny broth and GasSP with different w/v of Gas. The control was 0mg of GasSP, the treatment was 9mg of GasSP with 0%, 0.5%, 1%, 2% w/v Gas inside.)

It is apparent from this chart that between the 12th and 16th hour, there was a vigorous drop in the OD value of treatment group H; between the 13th and 16th hour, there was also a dramatic decline in the OD value of treatment group B, D and F (Figure 7 and 8).

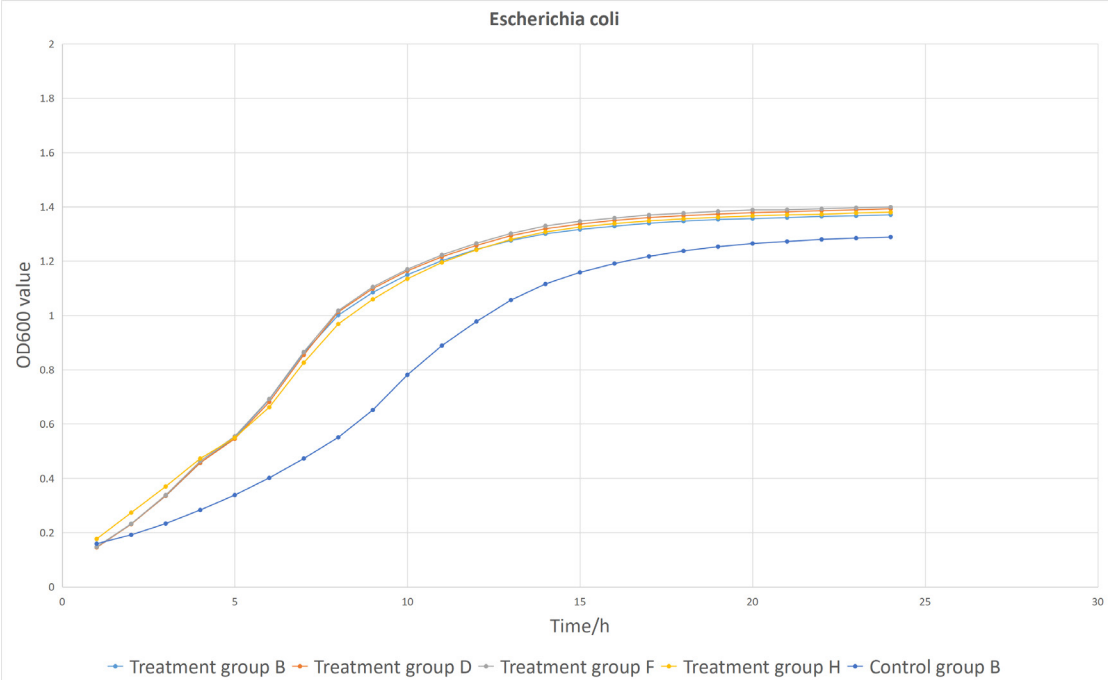

Figure 9. Bacterial growth curve of Escherichia coli

(Bacterial growth curve of Escherichia coli for 24h growth in the mixture of lysogeny broth and GasSP with different w/v of Gas. The control was 0mg of GasSP, the treatment was 9mg of GasSP with 0%, 0.5%, 1%, 2% w/v Gas inside.)

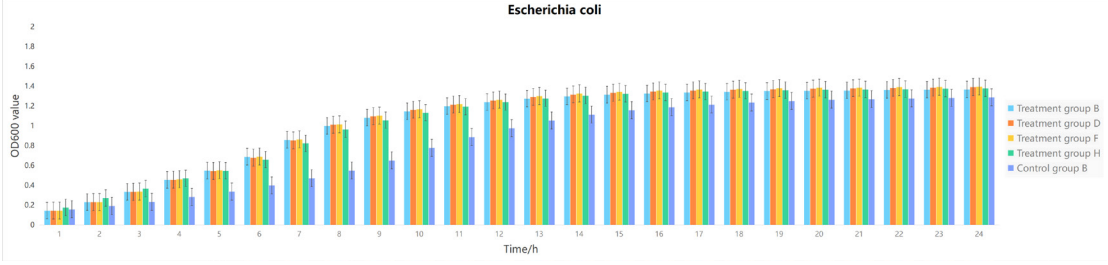

Figure 10. Standard error of mean (Mean±SEM) of OD values of Escherichia coli per hour between each groups

(Standard error of mean (Mean±SEM) of OD values of Escherichia coli per hour between each groups during 24h growth in the mixture of lysogeny broth and GasSP with different w/v of Gas. The control was 0mg of GasSP, the treatment was 9mg of GasSP with 0%, 0.5%, 1%, 2% w/v Gas inside.)

The most striking result to emerge from the data(Figure 7, 8, 9 and 10) is that all of the treatment groups were still more bacterial growth promoting compare to the control group H.

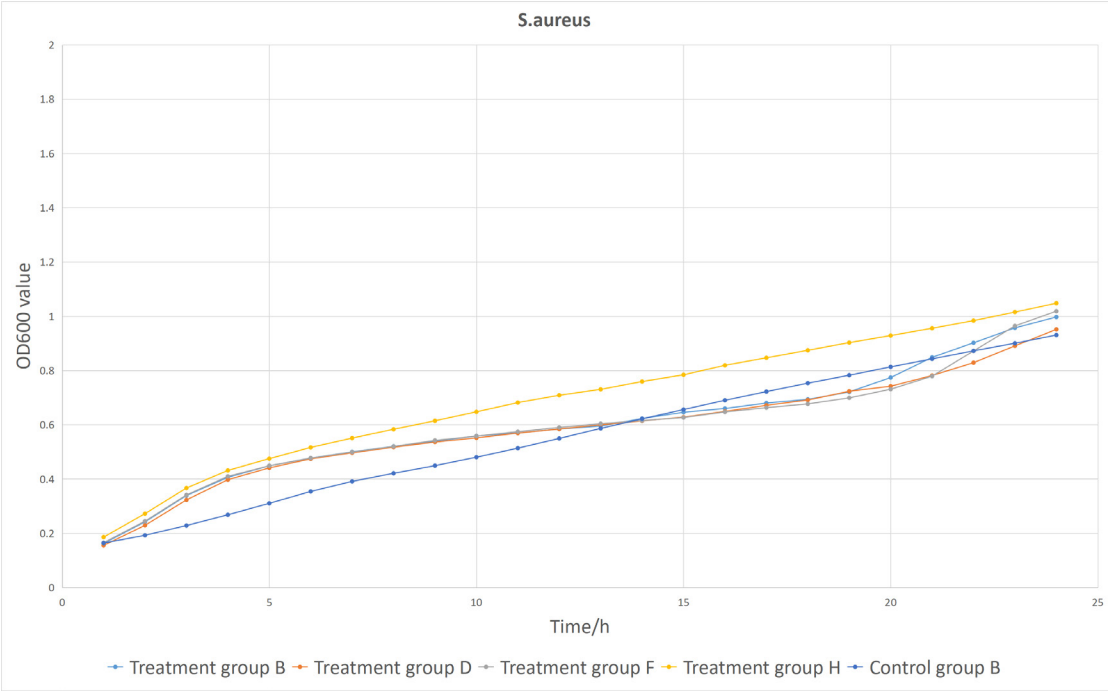

Figure 11. Bacterial growth curve of S.aureus

(Bacterial growth curve of S.aureus for 24h growth in the mixture of lysogeny broth and GasSP with different w/v of Gas. The control was 0mg of GasSP, the treatment was 9mg of GasSP with 0%, 0.5%, 1%, 2% w/v Gas inside.)

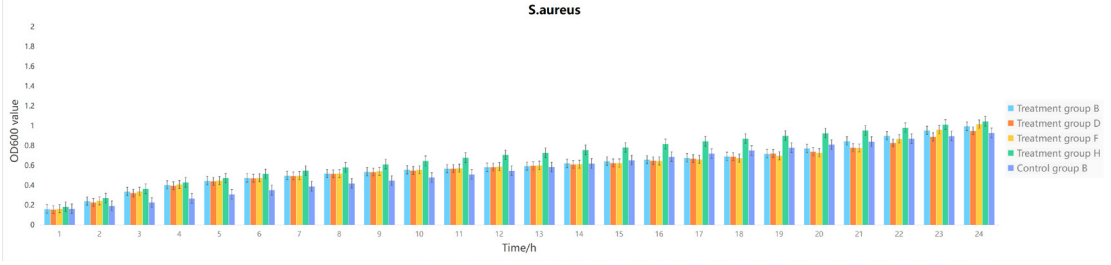

Figure 12. Standard error of mean (Mean±SEM) of OD values of S.aureus per hour between each groups

(Standard error of mean (Mean±SEM) of OD values of S.aureus per hour between each groups during 24h growth in the mixture of lysogeny broth and GasSP with different w/v of Gas. The control was 0mg of GasSP, the treatment was 9mg of GasSP with 0%, 0.5%, 1%, 2% w/v Gas inside.)

The bacterial growth curve of each treatment group were moderately unstable, and there was no correlation between the OD value and different variables of each group (Figure 11 and 12).

4.3 Experiment 3

4.3.1 Overview

This experiment aimed to test the antibacterial properties of GasSP without lyophilization with different Gas concentrations against P.aeruginosa, Escherichia coli and S.aureus.

4.3.2 Summary

No significant zone of inhibition for these three types of bacteria were found in the control and treatment groups.

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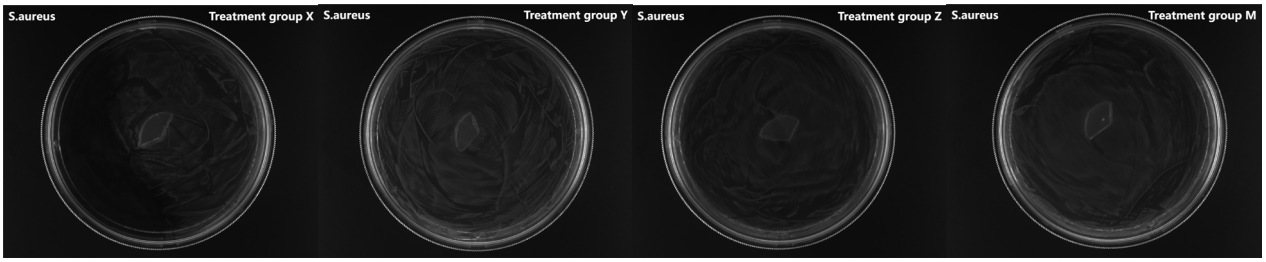

Figure 13. Zone of inhibition for S.aureus

(Zone of inhibition for S.aureus around GasSP without lyophilization with different concentration of Gas inside after 12h growth in the solid lysogeny broth(LB) medium. The control was GasSP with 0% w/v Gas inside, the treatment was GasSP with 0.5%, 1%, 2% w/v Gas inside.)

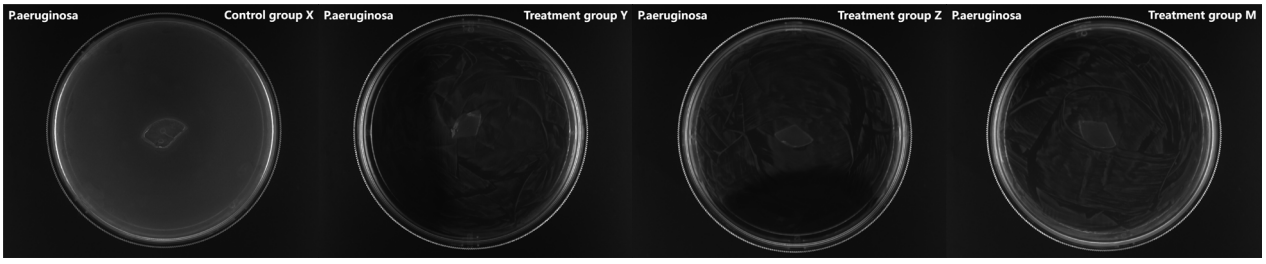

Figure 14. Zone of inhibition for P.aeruginosa

(Zone of inhibition for P.aeruginosa around GasSP without lyophilization with different concentration of Gas inside after 12h growth in the solid lysogeny broth(LB) medium. The control was GasSP with 0% w/v Gas inside, the treatment was GasSP with 0.5%, 1%, 2% w/v Gas inside.)

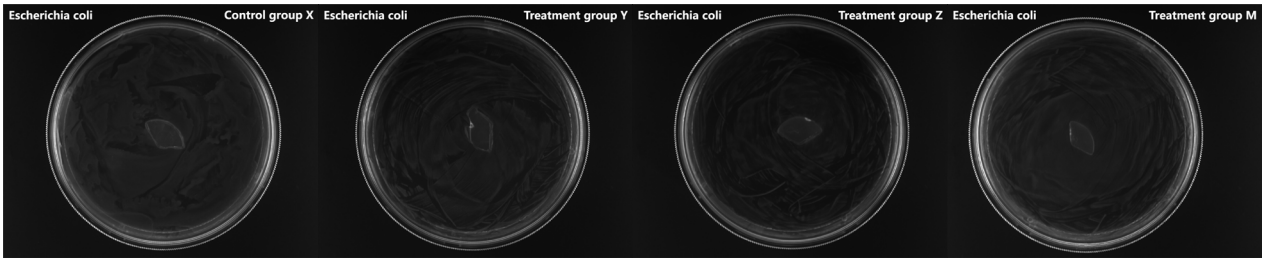

Figure 15. Zone of inhibition for Escherichia coli

(Zone of inhibition for Escherichia coli around GasSP without lyophilization with different concentration of Gas inside after 12h growth in the solid lysogeny broth(LB) medium. The control was GasSP with 0% w/v Gas inside, the treatment was GasSP with 0.5%, 1%, 2% w/v Gas inside.)

4.4 Experiment 4

4.4.1 Overview

This experiment aimed to test the antibacterial property of Gas with different concentrations against P.aeruginosa, Escherichia coli and S.aureus.

4.4.2 Summary

The results showed that Gas was antibacterial. There was a significant positive correlation between the concentration of Gas and the antibacterial properties of the solution under test against P.aeruginosa, Escherichia coli and S.aureus.

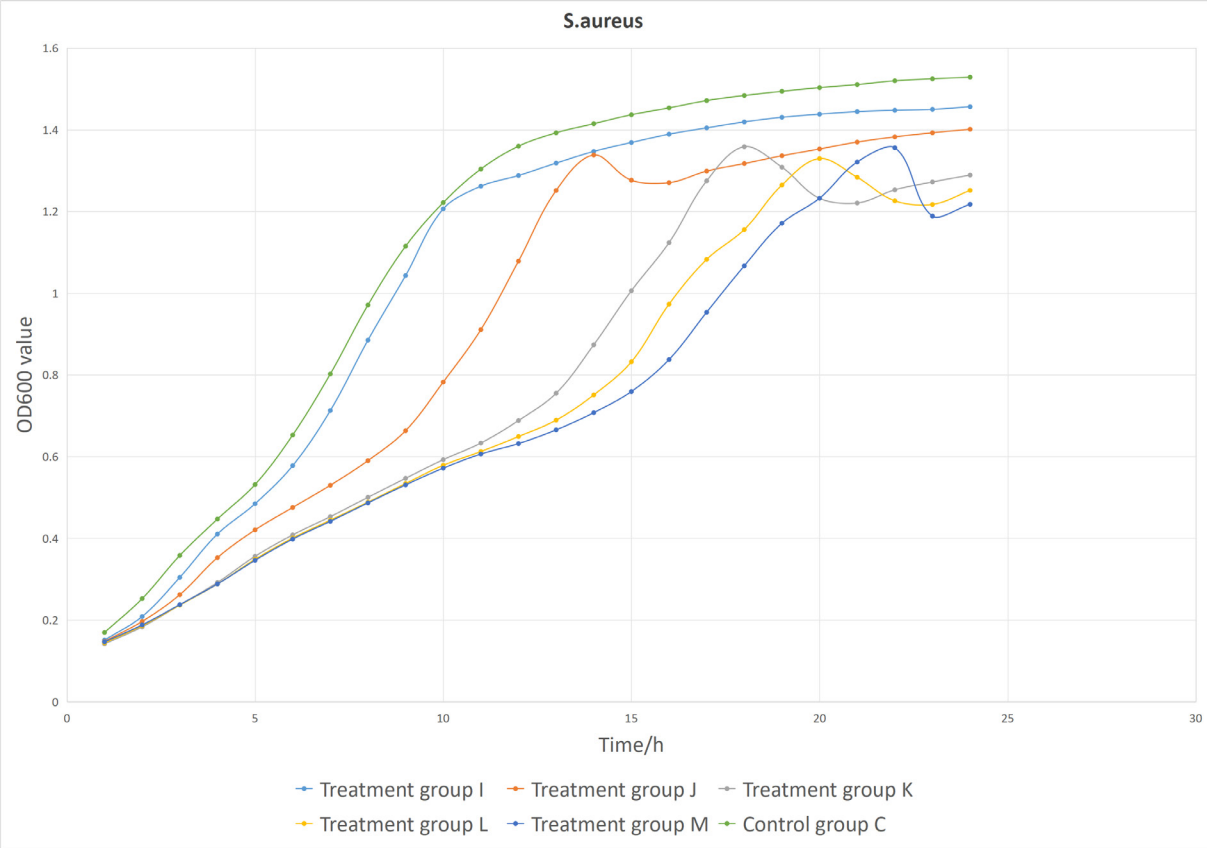

Figure 16. Bacterial growth curve of S.aureus

Bacterial growth curve of S.aureus for 24h growth in the mixture of lysogeny broth and Gas with different concentration. The control was 0mg of Gas, the treatment was 10mg, 20mg, 30mg, 40mg and 50mg of Gas.

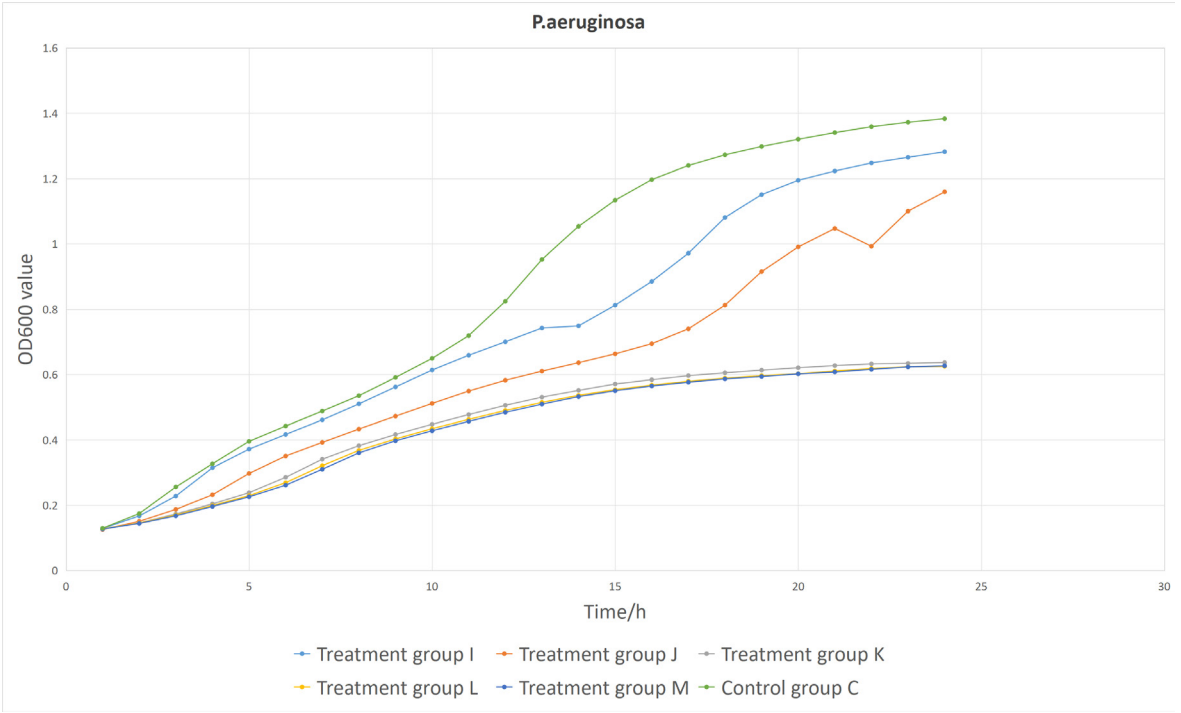

Figure 17. Bacterial growth curve of P.aeruginosa

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Bacterial growth curve of P.aeruginosa for 24h growth in the mixture of lysogeny broth and Gas with different concentration. The control was 0mg of Gas, the treatment was 10mg, 20mg, 30mg, 40mg and 50mg of Gas.

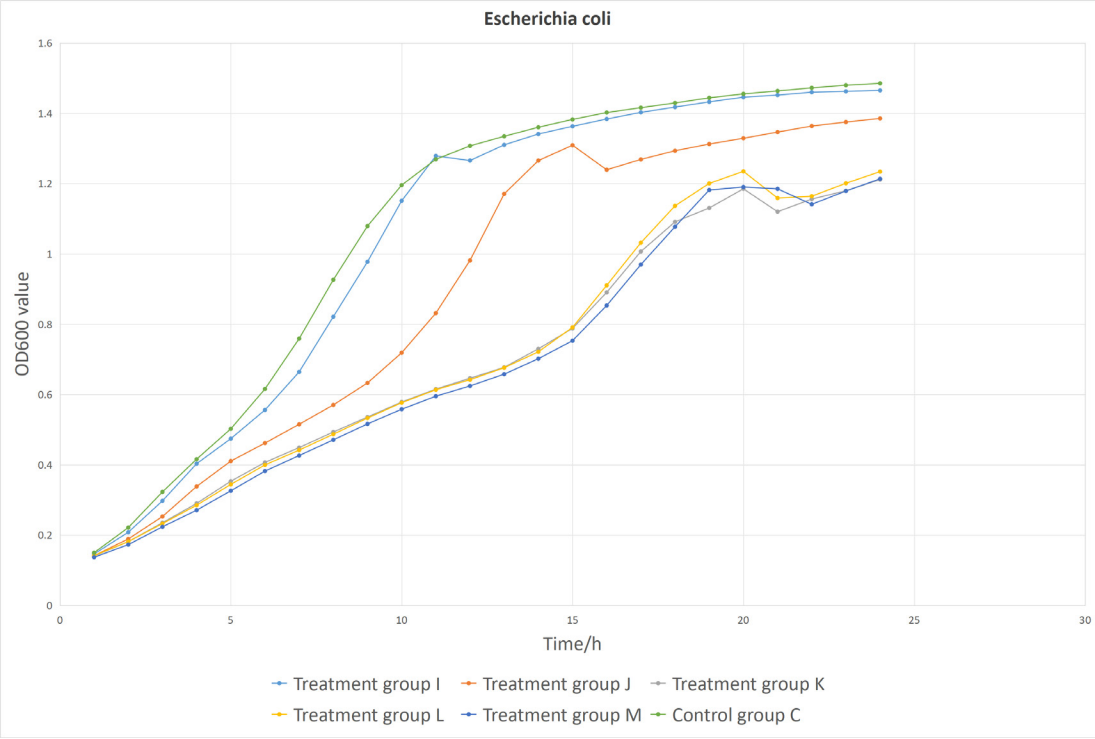

Figure 18. Bacterial growth curve of Escherichia coli

Bacterial growth curve of Escherichia coli for 24h growth in the mixture of lysogeny broth and Gas with different concentration. The control was 0mg of Gas, the treatment was 10mg, 20mg, 30mg, 40mg and 50mg of Gas.

4.5 Expert interview

The most striking result to emerge from the data of the experiment 1, 2 and 3 is that the significance difference between each group is dramatically small. Talking about this issue the interviewee said: 'Here are two possible explanations. First, if the experimental data was carried out with the correct process, and without mistakes, we should trust the data. And not to mention the antibacterial properties of GasSP is an exploratory discovery, there is no reference to the literature of the predecessors, then no matter how different the results are from what we expect, we should believe and respect the results, and develop and utilize them further. The second explanation is that there may have been a problem with the design of the experiment. The Gas concentration might be too low to reflect the slight difference of antibacterial properties in the OD value of GasSP. This leads to the difference in the OD values between each group that we got is just an experimental error.'

It was also conjectured that the reason why experimental results show that GasSP was slightly bacterial growth promoting might be that the effect of strontium and pectin combined to promote bacterial growth is stronger than the antibacterial ability of Gas. The interviewee totally agreed with this explanation.

The interviewee was also asked to suggest any possible applications of GasSP according to the experimental results. As the interviewee stated, considering that Gas is antibacterial, pectin is able to promotes bone tissue regeneration and hemostasis, and strontiumis is able to promotes osteoblasts and angiogenesis, we can put Gas on the outside of the hydrogel dressing, and put the pectin and strontium on the inside the hydrogel dressing. Therefore, for the outside layer of the hydrogel dressing, In addition to physically blocking bacteria, Gas can be used to chemically kill bacteria, which prevents wound infection. For the internal layer, pectin and strontium can promote wound healing.

5. Discussion and evaluation

As an exploratory discovery, this study began with the aim of assessing the feasibility of the potential application of GasSP and its main components by conducting antibacterial tests in vitro. The results of this study indicate that GasSP slightly promotes bacterial growth and the difference between different concentrations of GasSP was not

significant. The results also show that Gas was antibacterial, which was consistent with previous studies.

On the question of Glycyrrhizic acid ammonium salt (Gas), a derivative of glycyrrhizic acid [23], this study produced results that corroborate the findings of several previous studied in this field. It has been shown to have antibacterial properties. Oyama analyzed the strong antibacterial effect of glycyrrhetinic acid and its derivatives, which includes ammonium salt, on several strains of S. aureus [7]. This was further supported by Yamashita, who demonstrated that disodium succinoyl glycyrrhetinate, a derivative of glycyrrhetinic acid, has antibacterial activity against S.mutans and also reduces its virulence [24]. Collectively, the evidence gathered from these studies suggests that glycyrrhizic acid ammonium salt may have antibacterial properties. The results of this study indicated that Gas is indeed antibacterial against P.aeruginosa, Escherichia coli and S.aureus.

However, the results from experiment 1, 2 and 3 has not previously been reported cause the GasSP is a newly invented biomaterial in this field. The most creative and crucial finding in this study is that the significance difference between each group is significantly small, a possible explanation for this result is that the Gas concentration might be too small to reflect the difference of antibacterial properties in the OD value of GasSP. This leads to the difference in the OD values between each group that we got is just an experimental error. At the same time, contrary to expectations, it was reported in this study that the GasSP was slightly bacterial growth promoting, this result is difficult to explain, but it might relate to the fact that strontium and pectin are both cell growth promoting, while Gas is antibacterial, therefore the bacterial growth promoting ability of strontium and pectin might be stronger than the antibacterial ability of Gas, which leads to the GasSP is slightly more bacterial growth promoting.

This finding has important implication for developing the application of GasSP in the medical field. Considering that Gas is antibacterial, pectin is able to promotes bone tissue regeneration and hemostasis, and strontium is able to promotes osteoblasts [25] and angiogenesis [26]. GasSP has the potential to be utilized as a hydrogel wound dressing, which are structured as follows: putting Gas on the outside layer of the hydrogel dressing, and putting the pectin and strontium on the inside layer of the hydrogel dressing. As a result, the outside layer of the hydrogel dressing is able to physically blocking bacteria, and the Gas in the outside layer can be used to chemically kill bacteria, which prevents wound infection. For the internal layer, pectin and strontium can promote wound healing at the same time.

Futher study must be conducted to determine whether or not the GasSP is antibacterial after increasing the concentration of Gas inside the GasSP, in order to figure out whether or not the little significance difference between each group was caused by the low Gas concentrations inside the GasSP. More information on GasSP, strontium, pectin and Gas would help to establish a greater degree of accuracy in the feasibility of the application of GasSP as a wound dressing.

There are two major possible limitations in this study that could be addressed in future research. First, the study focused on a completly new hydrogel material, this has leaded to a lack of publicated literatures related to the GasSP, however, this situation can be used as an opportunity to develop the research gap. Therefore this paper put forward the conjecture and suggestions for the future research direction of the relevant field. Second, due to the submission period of the paper, the time of the experimental investigation is limited to a certain extent, resulting in the study only obtained first-hand data on the antibacterial properties of the GasSP and Gas, the rest of the properties of the research are derived from second-hand data. This may lead to insufficient relevance and accuracy of the data.

Overall, this study demonstrates that GasSP as a newly developed material is very likely to be used as a skin dressing in clinics. At the same time, the study found that biomaterials that promote bacterial growth are significantly underestimated. Many of the current literature prefers to study antibacterial dressings. But in fact, dressings that promote bacterial growth can also be used to microbes to treat skin diseases, and so on. This finding is able to serve a great basis for future studies in biomaterial.

6. Conclusion

This paper provides a description of the antibacterial properties of GasSP and its component, and the potential application of GasSP.

This project was undertaken to design several antibacterial test of GasSP and Gas against P.aeruginosa, Escherichia coli and S.aureus. And evaluate the feasibility of the conjectured application of GasSP as a skin dressing. The following conclusions can be drawn from the present study. Firstly, there was no significant difference between each group of GasSP with different w/v of Gas. In addition the result shows that the GasSP with different concentration of Gas inside are slightly bacterial growth promoting against P.aeruginosa, Escherichia coli and S.aureus. What‘s more, It is also demonstrated by this study that the Gas is antibacterial, and there is a positive correlation between the concentration of Gas and the antibacterial property of Gas. According to this result from the experiment, It is expected that The GasSP can be used as a component

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in the skin wound dressing, to be more specific, Gas can be put into the outer layer of the skin dressing and pectin and strontium can be put into the inside layer of the skin dressing, so that the skin dressing can physically block bacteria as well as chemically kill the bacteria, at the same time, pectin and strontium are able to promote the growth of damaged cells.

Overall, these findings enhanced our understanding of the antibacterial property of Gas, and suggest that the application of GasSP will play an important role in the field of biomaterial.

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