Antimicrobial activity of chitosan nanoparticles loaded with 0.7% tetracycline against Porphyromonas gingivalis

Martina Amalia,* Irma Ervina, Silvia, Erdi E. Nasution

Abstract

Objective: Nanoparticle technology has also come to the fore as a viable drug delivery strategy, providing opportunities for controlled release, protecting the active ingredient from enzymatic or environmental degradation, and local retention. Porphyromonas gingivalis is a Gram-negative obligate anaerobe rod involved in the etiology of periodontal disease. Tetracycline has been widely used as an adjuvant in periodontal therapy due to its antibacterial efficacy of these drugs.

Material and Methods: Samples of this study were the pure culture of P. gingivalis (ATCC 33 277) in Mueller Hinton Agar (MHA). The ion gelation method made a Chitosan nanoparticle loaded with 0.7% tetracycline. Chitosan nanoparticles loaded with 0.7% tetracycline were directly transferred to the microbiology laboratory to maintain the stability of the material. Mueller Hinton Agar media inoculated with bacteria had holes to input chitosan nanoparticles loaded with 0.7% tetracycline. After repeating these operations four times, the incubator was incubated at 37 °C for 48 hours. Calipers were used to observe and measure the diameter of the bright zone (clear zone) formed around the hole.

Results: The result shows the potent antimicrobial effectiveness of chitosan nanoparticles loaded with 0.7% tetracycline against P. gingivalis in vitro.

Conclusion: This research has shown a potent antimicrobial activity of chitosan nanoparticles loaded with 0.7% tetracycline against P. gingivalis in vitro.

Keywords: Chitosan nanoparticle, Porphyromonas gingivalis, Tetracycline

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Introduction

Porphyromonas gingivalis is involved in the etiology of periodontal disease. P. gingivalis is a gram-negative, obligate anaerobe rod that produces black-brown colonies on anaerobic blood agar. The oral cavity is found mainly immersed in the subgingival microflora. It is considered a pathogen: it stimulates the host's immune response, evades defense mechanisms, and destroys host tissues by secreting its substances.1

Tetracycline has been widely used as an adjuvant for periodontal treatment due to its antibacterial activity of these drugs. Their ability to inhibit host-derived matrix metalloproteinases and bone resorption in organ culture has also been invoked as a therapeutic rationale.2 However, the emergence of antibiotic resistance and new pathogenic strains and a lack of appropriate therapeutics have led to infections that remain a significant cause of disease and mortality in modern societies. There has been a growing interest in using new agents, such as antimicrobial polymers, as alternatives for therapy and disinfection.3

Chitosan, a biopolymer of marine origin, has recently attracted attention due to its significant antimicrobial properties and its advantages as non-toxic, biodegradable, and biocompatible.3-4 It has been applied in various fields, including biotechnology, medicine as a drug delivery system, tissue engineering, and cosmetics.4 Recently, chitosan derived from shrimp has been recognized as a Generally Recognized As Safe (GRAS) for general use in foods by the Food and Drug Administration.5-6 In addition, Japan and Korea have approved chitosan as a food additive since 1983 and 1995, respectively.6 Chitosan is used for the transmucosal delivery of peptides and proteins because it is mucoadhesive and can open tight connections between epithelial cells. Consequently, chitosan facilitates the transport of macromolecular drugs.4

Several factors, such as pH, influence chitosan's antimicrobial activity, as it is only soluble under acidic conditions (pH<6). In this regard, modifications to the three-dimensional structure of chitosan allow the compound to solubilize at neutral pH and maintain its antimicrobial activity.3-4 De Paz et al.7 generated nanoparticles by ion gelation with sodium tripolyphosphate. Sodium tripolyphosphate (TPP) has been given the classification of GRAS, which indicates that it is considered safe to add chitosan to foods and beverages.4
Table 1. The diameter of the inhibition zone of chitosan nanoparticles loaded with 0.7% tetracycline against Porphyromonas gingivalis in four repetitions.

<table>
<thead>
<tr>
<th>Repetition</th>
<th>Diameter of Inhibition Zone (mm)</th>
<th>Average</th>
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<tr>
<td>I</td>
<td>33</td>
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<tr>
<td>II</td>
<td>32</td>
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<td>III</td>
<td>33.5</td>
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<tr>
<td>IV</td>
<td>32.8</td>
<td>32.8</td>
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Figure 1. Inhibitory zone of chitosan nanoparticles loaded with 0.7% tetracycline against Porphyromonas gingivalis in (a) first repetition, (b) second repetition, (c) third repetition, and (d) the fourth repetition.

Chitosan was employed as a nanocarrier to deliver both synthetic and natural substances to potentiate or modulate their antimicrobial activity. Various classes of antibiotics are encapsulated in chitosan nanoparticles, increasing the drug’s effectiveness against bacterial growth. In the current study, we observe the antimicrobial activity of chitosan nanoparticles loaded with 0.7% tetracycline against P. gingivalis in vitro.

Material and Methods

This experimental study was conducted at the Laboratory of Microbiology, Universitas Sumatera Utara Hospital. This research was validated by Animal Research Ethics Committees/AREC Universitas Sumatera Utara with the number No.0383/KEPH-FMIPA/2021. Samples of this study were the pure culture of P. gingivalis (ATCC 33 277) in Mueller Hinton Agar (MHA). The number of repetitions in this study was four times.

Chitosan nanoparticles loaded with 0.7% tetracycline were made with the ionic gelation method at room temperature. Chitosan was diluted in 1% acetic acid and stirred for 24 hours, and pH was adjusted to 5.5 by adding 0.01 N NaOH. Sodium tripolyphosphate (TPP) was then added to the solution under stirring. The final solution was homogenized for 1 minute, and tetracycline (Kimia Farma) was rushed to the final solution for another 20 minutes. Chitosan nanoparticles loaded with 0.7% tetracycline were directly transferred to the microbiology laboratory to maintain the stability of the material.

The bacterial growth medium was prepared in 40 petri (20 mL/petri) using 12 g of powdered Mueller-Hinton dissolved in 240 mL of distilled water, reheated on a stove, and magnetically boiled. Then the media was sterilized in an autoclave for 15 minutes by air pressure at 2 atm at a temperature of 121 °C, reheated to a boil, poured into petri, and allowed to cool. Activity breeding specimens were performed in an anaerobic atmosphere in a CO2 incubator.

Porphyromonas gingivalis used was a stem-cell specimen bred purely on media MHA, prepared in the previous procedure in an anaerobic atmosphere. A total of 12 pure cultures were cultured and suspended using 0.9% NaCl solution to give McFarland 0.6 turbidity as a standard for bacterial counts 1 x 106 CFU / ml. P. gingivalis was taken from MHA near the bunsen so as not to be contaminated. Mueller Hinton Agar media inoculated with bacteria had holes to input chitosan nanoparticles loaded with 0.7% tetracycline. After repeating these operations four times, the incubator was incubated at 37 °C for 48 hours. Calipers were used to observe and measure the diameter of the bright zone (clear zone) formed around the hole.

Results

The zone diameter of inhibition was measured to determine the efficacy of the tetracycline gel against the bacteria tested. The clear inhibition zone is a circular area that shows no bacterial growth in the surrounding area of drugs. The wider the circle diameter clear zone, the greater the zone of inhibition figure 1.

The inhibition zone of chitosan nanoparticles loaded with 0.7% tetracycline against P. gingivalis measures the diameter of the bright zone (clear zone) formed around the hole with the caliper.

Table 1 shows the average inhibition zone of chitosan nanoparticles loaded with 0.7% tetracycline against P. gingivalis obtained from this study was approximately 32.8 mm.
Discussion

The average inhibition zone of chitosan nanoparticles loaded with 0.7% tetracycline against P. gingivalis obtained from this study was approximately 32.9 mm. Davis and Stout said that the suppression zones less than 5 mm formed in the agar diffusion test were classified as weak, the 510 mm suppression zones were classified as average, and the 1019 mm and 20 mm were classified as strong or very strong. Based on these statements, nano chitosan loaded with 0.7% tetracycline has a powerful antibacterial effect.9

Our findings promise chitosan nanoparticles as an alternative antimicrobial agent for treating bacterial infections. Chitosan has a wide range of antibacterial activity, but it has various effects on microorganisms. The minimum inhibitory concentration (MIC) ranged from 10 to 5000 mg/L for fungi. Devlieghere et al. tested the antimicrobial activity of chitosan with an Mw of 43 kDa and a DA of 94%.4 It was observed that gram-negative bacteria were less resistant while the effect on gram-positive bacteria varied.5

Several possibilities have been described for the mechanism of action of chitosan, from bacterial binding to DNA to mRNA inhibition to interaction with surface molecules. The ability of chitosan to bind DNA has generally been studied for gene delivery. Still, the contribution of such capacity in antimicrobial activity is unclear because chitosan would not reach a target in the cytoplasm. Another hypothesis was that chitosan binds to the bacterial membrane and perturbs it. However, one study reported that hydrated chitosan is too big to cross the cell wall and interact directly with the cell membrane.4

The widely accepted hypothesis is that the polycationic nature of chitosan is due to the presence of amine groups (NH3+) of glucosamine and might be a key feature of its ability to interact with negatively charged surface components of many microorganisms, causing extensive alterations to the cell surface, leading to leakage of intracellular substances that result in cell death.9 In this case, lipopolysaccharide in gram-negative bacteria and teichoic acid in gram-positive bacteria play a significant role in binding chitosan. The alteration and destabilization of cell membrane function result from the disruption of cell wall dynamics.4 A study published by Leon et al. demonstrated that the antimicrobial activity of chitosan microparticles was exerted by binding the microparticles to the bacteria.6

Evaluated the efficacy of cefazolin-loaded chitosan nanoparticles in opposition to multi-resistant gram-negative microorganisms consisting of E. coli, K. pneumonia, and P. aeruginosa. Chitosan nanoparticles loaded with the active ingredient showed antibacterial activity against three microorganisms.10 Similarly, the efficacy of drug-loaded chitosan nanoparticles against antibiotic-resistant bacterial strains was also demonstrated for vancomycin against drug-resistant S. aureus.11 Sebastian J et al.12 in research Roselle flower petals extract is effective at inhibiting F. nucleatum and P. gingivalis biofilms. This anti-biofilm agent may be developed as an alternative therapy for preventing periodontal disease.12,13 In this research chitosan nanoparticles loaded with 0.7% tetracycline showed antibacterial activity against P. gingivalis.

Conclusion

This research has shown a potent antimicrobial activity of chitosan nanoparticles loaded with 0.7% tetracycline against Porphyromonas gingivalis in vitro with an inhibitory zone of 33 mm. This research may be the basis for further research to enable later clinical use of chitosan nanoparticles loaded with 0.7% tetracycline to support periodontal treatment.

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Conflict of Interest

The authors report no conflict of interest

References

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