

# The effect of coating chitosan on *Porphyromonas gingivalis* biofilm formation in the surface of orthodontic mini-implant

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## ABSTRACT

Infection is the main problem for the failure of orthodontic mini-implant. Modern prevention of infection is now focused on local antibacterial coatings on implant devices. Chitosan is biocompatible and has antibacterial properties. Azithromycin is a synthetic antibiotic with immunomodulatory properties in which it has an advantage over the rest of antibiotics. This study aimed to evaluate the effect coating chitosan on the orthodontic mini-implant in *Porphyromonas gingivalis* biofilm formation. This is an experimental study using 25 orthodontic mini-implants. Five samples were coated with chitosan, 5 samples were coated with chitosan–azithromycin, 5 samples were coated with azithromycin, 5 samples were uncoated, and 5 samples were uncoated and were not exposed to *P. gingivalis*. *P. gingivalis* biofilms on the surface of the orthodontic mini-implant were observed after 24 h of incubation. *P. gingivalis* biofilm mass inhibition was highest in the azithromycin-treated group, followed by chitosan + azithromycin and chitosan only. The one-way ANOVA statistic test and *post hoc* Bonferroni statistic test of *P. gingivalis* biofilm mass show a significant difference between and within groups of experiments ( $P < 0.05$ ). The Pearson correlation test with a value of  $R = +0.88$ , indicated that the bacterial viability count and the biofilm mass have a strong positive correlation. In conclusion, orthodontic mini-implant coated with chitosan, chitosan with azithromycin, or azithromycin only effectively suppressed *P. gingivalis* biofilm formation.

**Key words:** Azithromycin, biofilm, chitosan, coating of orthodontic mini-implant, *Porphyromonas gingivalis*

## INTRODUCTION

Orthodontic mini-implants are widely used in the last decade.<sup>[1]</sup> Several studies have revealed that the orthodontic

mini-implant was stable enough against orthodontic forces of 50–250 g.<sup>[2]</sup> Several other studies also stated that after insertion, orthodontic mini-implants experienced mobility and then dislodged even before administering orthodontic forces.<sup>[3]</sup> A failure rate of 6.6%–16.1% from orthodontic mini-implants is a greater failure rate than dental implants or other anchoring devices such as miniplate (2.6%–7.3%).<sup>[4]</sup> Mechanisms that cause the orthodontic mini-implants to fail are still unclear.<sup>[5]</sup> Several factors considered to play some roles in the failure of orthodontic mini-implant are

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age, smoking habits, oral hygiene, cortical bone thickness, insertion angle, direction and magnitude of a force, and bacterial contamination.<sup>[6]</sup>

A previous study stated that the main factor causing implant overdenture's failure is the colonization of the implant surface by pathogenic bacteria.<sup>[7]</sup> Inflammation of the tissue around the orthodontic mini-implant is believed to be associated with an increase of 30% in orthodontic mini-implant failure.<sup>[8]</sup>

Bacteria adapt better to the unfavorable external conditions due to the antimicrobial's inability to penetrate the multilayered biofilm matrix and the increase of the antibacterial resistant gene expression.<sup>[9]</sup>

*Porphyromonas gingivalis* is the most dominant late colony in peri-implantitis and periodontitis. Peri-implantitis is a progressive and irreversible inflammation in the tissue surrounding the implant body accompanied by bone loss, decreased osseointegration, and is one of the main risk factors for implant overdentures' failure.<sup>[10]</sup>

One management for peri-implantitis is administering antibiotics either systemically or locally, which must be done wisely and rationally considering bacterial resistance's rapid development.<sup>[11]</sup> The latest approach to counter bacterial resistance is to use a natural antibacterial originating from nature.<sup>[12]</sup> One plausible approach considered to be the most effective way to prevent bacterial growth in the implant's tissues is to cover the implant surface with a natural antibacterial coating that also bears biodegradable properties.<sup>[13]</sup> The antibacterial coating is expected to prevent bacterial attachment on the implant surface, thus preventing biofilm formation.<sup>[14]</sup>

Azithromycin is effective against Gram-negative bacteria, can penetrate biofilms, and is also believed to be very effective against *P. gingivalis* strain.<sup>[15,16]</sup> Another beneficial feature of azithromycin is its ability to increase body immunity, which is why this antibiotic received considerable attention in the last decade.<sup>[17]</sup>

Chitosan biopolymer is a natural product that currently has received considerable attention from researchers due to its biocompatibility, bioadhesive, bacteriostatic, and osteoconductive properties.<sup>[18,19]</sup> These properties make chitosan now widely researched in the medical world as a wound-healing agent,<sup>[20]</sup> drug delivery agent,<sup>[21]</sup> food preservatives,<sup>[22]</sup> and also as a coating agent for orthopedic and dental implants.<sup>[23]</sup>

The efficacy of chitosan antibacterial coating on titanium implants has been widely published in orthopedic and denture implant researches.<sup>[24,25]</sup> Here, we evaluate the effect of chitosan and azithromycin's orthodontic mini-implants on *P. gingivalis* biofilm.

## MATERIALS AND METHODS

This study was an *in vitro* experimental study. The samples' size was calculated using Federer's sample size estimation formula for unpaired numerical data with normal data distribution. Using Federer's formula, we obtain the number of samples of  $n > 4.75$ , rounded to 5 samples. Thus, this study will use 5 samples for each treatment group, i.e., coated with chitosan (Sigma-Aldrich, Germany), chitosan + azithromycin (Pfizer, USA), azithromycin, without any coating, and without any coating or cultured in *P. gingivalis* bacteria (negative control group).

### Inclusion criteria

The orthodontic mini-implants used in this study are the mini-implants that have fulfilled the inclusion criteria. The surface of orthodontic mini-implants is made rough with 80 grit sandpaper. The samples are then rinsed with distilled water and ethanol and then dried. After the samples are dry, the mini-implants are sterilized in an autoclave. The sampling was done by random sampling technique.

### Bacterial preparations

*P. gingivalis* was cultured in a Trypticase soy broth media. Bacterial suspension concentration of  $10^6$  CFU/ml was prepared in 5 ml per tube for 4 treatment sample groups (1 group – a negative control which was not exposed to bacteria).

### Chitosan preparation

Chitosan solutions were prepared at a concentration of 2%–1% acetic acid.<sup>[22]</sup> Each sample will use 4 ml of this solution. The mixture of chitosan and azithromycin solutions containing 20 ml of 0.5 µg of azithromycin was prepared. All of the solutions used for this study were sterilized using a syringe filter 22 µm.

The biofilm formation in the surface orthodontic mini-implants was rinsed with phosphate-buffered saline two times in order to remove nonsticking bacteria, they were then dried and dissolved the biofilm in the surface of the mini-implant by 96% ethanol solution, and finally, the ethanol elution optical density was measured using ELISA reader (Metertech M965+).

## RESULTS

The results showed that *P. gingivalis* biofilm mass inhibition was highest in the azithromycin-treated group, followed by chitosan + azithromycin and chitosan only [Figure 1].

*Post hoc* Bonferroni statistic test of *P. gingivalis* biofilm mass shows that there is a significant difference between and within groups of experiments ( $P < 0.05$ ) [Table 1].

The viability of *P. gingivalis* in biofilm was also evaluated, and the result shows that in the azithromycin-treated group, *P. gingivalis* was comparable to the

chitosan + azithromycin-treated group even though in the azithromycin group was the lowest one. The inhibitory effect of the chitosan-only-treated group was lower compared to chitosan + azithromycin and azithromycin [Figure 2].

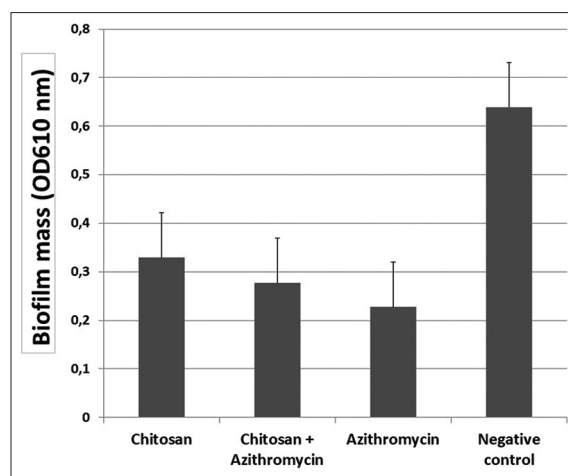
*Post hoc* Bonferroni statistic test of *P. gingivalis* viability in biofilms shows that there is a significant difference between and within groups of experiments ( $P < 0.05$ ) [Table 2].

In this study, both the viability of *P. gingivalis* in biofilm and biofilm mass quantification indicate a similar result, as indicated by the Pearson correlation test with a value of  $R = + 0.88$ , which means that the bacterial viability and the biofilm mass have a strong linear positive correlation [Figure 3].

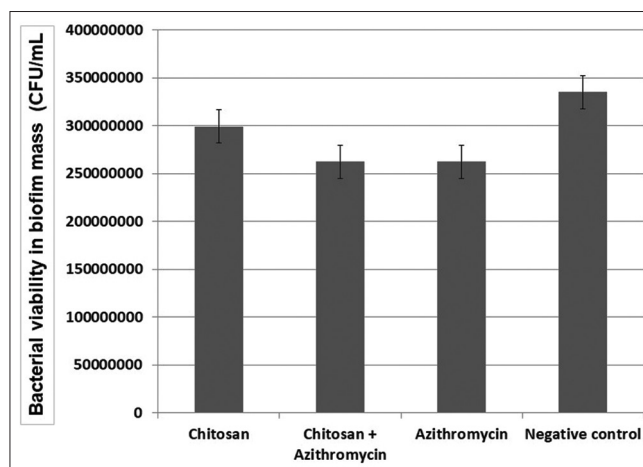
## DISCUSSION

Our study showed that *P. gingivalis* biofilm mass inhibition was highest in the azithromycin-treated group, followed by chitosan + azithromycin chitosan only. This result is quite obvious, considering that azithromycin is very effective against *P. gingivalis* bacteria.<sup>[26]</sup> Besides, the absence of a drug delivery system will cause the antibiotics to release the substrate as quickly as possible to the infected tissue area.<sup>[27]</sup> Antibiotics without a drug delivery system will release the antibiotic substrate by 65% at the first 1 min and 95% at 5 min; this pattern is advantageous in laboratory studies, however, clinically, it is not necessarily beneficial, because antibiotics cannot fight bacteria for a long time, whereas the incidence of peri-implantitis can occur several days or even weeks after implant placement.<sup>[28]</sup>

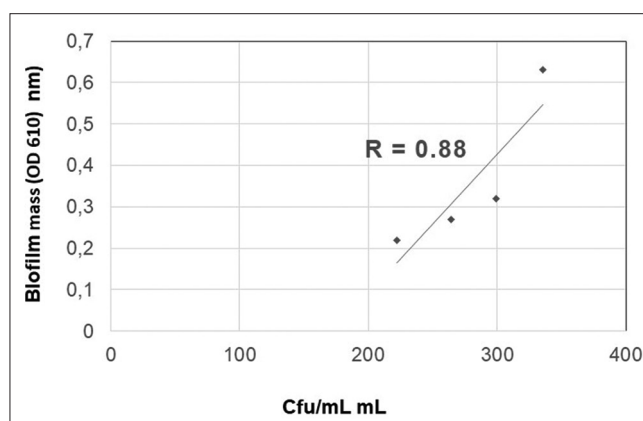
A previous study reported that the antibacterial effectiveness of chitosan depends on several factors, i.e., microbial factors such as the species and numbers, chitosan's intrinsic factors (molecular weight, concentration, and deacetylation level), physical factors such as chitosan solubility in the water, and environmental factors, i.e., acidity (pH), temperature, and reaction time.<sup>[29]</sup> Thus, it can be safely considered that chitosan biomaterials used in this study have met the ideal criteria. There are pros and cons regarding the antibacterial effectiveness of chitosan against Gram-negative bacteria; Raafat *et al.*,<sup>[30]</sup> in their study, suggested that chitosan is less effective against Gram-negative bacteria; meanwhile, İkinci *et al.*<sup>[31]</sup> stated that chitosan is effective against anaerobic Gram-negative bacteria *P. gingivalis*. The results of this study are in line with the results of İkinci *et al.*, i.e., chitosan can inhibit the growth of *P. gingivalis*. However, the antibacterial effectiveness of the chitosan group in this study was lower than the azithromycin group and the combination of chitosan–azithromycin. This phenomenon may happen because the antibacterial properties of chitosan are bacteriostatic by destabilizing the cell membrane without killing bacteria.<sup>[32-34]</sup> Thus, the number of *P. gingivalis* colonies formed on the implant surface does not necessarily decrease because what happens is only a decrease in the bacteria's virulence only.<sup>[35]</sup>



**Figure 1:** The *Porphyromonas gingivalis* biofilm mass of the treated group and the negative control



**Figure 2:** The viability *Porphyromonas gingivalis* in biofilm mass of the treated group and the negative control



**Figure 3:** The correlation between the optical density (610) and colony count (CFU/mL) of *Porphyromonas gingivalis* in biofilm in the surface of mini-implant orthodontic

The group in which the orthodontic mini-implants were coated with the chitosan–azithromycin combination resulted in an antibacterial potency below the azithromycin group.

**Table 1: Post hoc Bonferroni statistic test of *Porphyromonas gingivalis* biofilm mass**

The comparison between groups	P
Control versus chitosan	<0.05
Control versus azithromycin	<0.05
Control versus chitosan + azithromycin	<0.05
Chitosan versus azithromycin	<0.05
Chitosan versus chitosan + azithromycin	<0.05
Azithromycin versus chitosan + azithromycin	<0.05

**Table 2: Post hoc Bonferroni statistic test of *Porphyromonas gingivalis* viability count (CFU/mL)**

The comparison between groups	P
Control versus chitosan	<0.05
Control versus azithromycin	<0.05
Control versus chitosan + azithromycin	<0.05
Chitosan versus azithromycin	<0.05
Chitosan versus chitosan + azithromycin	<0.05
Azithromycin versus chitosan + azithromycin	<0.05

This result is probably due to one of the properties of chitosan that acts as a slow-release drug delivery agent/controlled delivery drug agent, where chitosan will deliver the drug in controlled conditions so that the drug will have a long therapeutic effect.<sup>[21]</sup> Norowski, in his study, has stated that a combination of antibiotics and chitosan on implant coating will produce a uniform drug delivery rate for 7 days, after which it continues to decline until the 4<sup>th</sup> week.<sup>[36]</sup> Another study by Greene *et al.* suggests that the combination of chitosan and gentamycin produced a uniform drug delivery rate until day 4.<sup>[29]</sup> Patel in his study also stated that chitosan with a combination of bioactive glass would deliver ampicillin until the 11<sup>th</sup> week.<sup>[37]</sup> It can be concluded that the combination of chitosan with antibiotics would slow down the rate of delivery of antibiotics to the intended area.<sup>[37]</sup> However, this is a desirable result regarding the implant coating with chitosan and antibiotics; thus, antibiotics will have the maximum efficacy and time to act in the tissues and minimize tissue toxicity risk.<sup>[28]</sup>

The increasing incidence of bacterial resistance and the scarcity of antibiotic inventions have changed the medical world's paradigm against infection.<sup>[12]</sup> The old paradigm is all about how to kill bacteria. In contrast, the new paradigm now leads to the use of biomaterials that were believed not to cause any bacterial resistance thanks to their function of only weaken the bacterial virulence by inhibiting the toxin production, blocking the bacterial nutrition, and increasing the host immunity.<sup>[38]</sup> The further preclinical study is needed in order to confirm whether chitosan only can prevent biofilm formation.

## CONCLUSIONS

We conclude that coating chitosan or combined with azithromycin is suppress *in vitro* the biofilm formation *P. gingivalis* in the surface mini-orthodontic implant.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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