



ORIGINAL ARTICLE

Chitosan gel prevents the growth of *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* in mini-implant during orthodontic treatment

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Abstract *Aims:* We evaluated the effect of chitosan gel on total oral bacteria, *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*, during orthodontic treatment with mini-implants.

Material and methods: Thirty subjects with 52 orthodontic mini-implants were divided into three groups: one group was treated with chitosan gel, the other group with chlorhexidine gel, and the control group with placebo. The plaque of the orthodontic *peri*-mini-implant area was collected before and after gel treatment. The total oral bacteria and *red-complex* bacteria of *P. gingivalis*, *T. forsythia*, and *T. denticola* were determined with reverse transcription-quantitative PCR.

Results: Thirty-four orthodontic mini-implants (65.38%) appeared as healthy and showed no clinical signs of inflammation. The total number of bacteria was reduced after chitosan gel application. The highest decrease in the proportion of *P. gingivalis* was observed in the chlorhexidine gel

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application group, which showed a value of 70.86%, whereas the chitosan gel application showed a reduction of only 26.59%, and the control gel application showed the lowest reduction effect of only 2.55%. The difference in the reduction between gel application groups was significant ($P < 0.05$) for *T. denticola* and *T. forsythia*.

Conclusion: The gel containing chitosan reduced the levels of total oral bacteria and *red-complex* bacteria.

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1. Introduction

Orthodontic mini-implants have been widely used in orthodontic science because of their advantages, such as resistance or strength to withstand the opposing forces generated by antagonistic teeth. Orthodontic mini-implants can also be inserted without the need for patient cooperation, and the cost is relatively low. The placement of orthodontic mini-implants is adjusted according to the biomechanics required, their insertion is minimally invasive, and they can be removed (Miyawaki et al., 2003, Roncone, 2011). However, orthodontic mini-implants can lead to some complications related to infection, such as peri mucositis and *peri-implantitis*. This condition begins when inflammation occurs in the tissue around the orthodontic mini-implant neck in contact with the buccal mucosa due to bacterial growth. Inflammation can be prevented by reducing bacterial contamination on orthodontic mini-implants (Oltamari-Navarro et al., 2009, Nagappan and John, 2012). Chlorhexidine is the most widely used antibacterial and plaque control agent because it effectively reduces plaque accumulation. Chlorhexidine shows antibacterial activity against gram-positive and gram-negative bacteria and is particularly suitable for treating and preventing oral tissue infections (Russell and Day, 1993, Oltamari-Navarro et al., 2009). Chlorhexidine in the gel was reported to have a more prolonged antibacterial effect than the chlorhexidine solutions, as demonstrated by Paolantonio et al. (Ikono et al., 2012, Nagappan and John, 2012).

Scientists have developed a biopolymer material, known as chitosan, for use in dentistry (Dutta et al., 2004, Bachtiar et al., 2016, Ikono et al., 2019a,b). Chitosan is derived from shrimp shells and obtained through partial and full alkaline deacetylation processes and by combining organic and inorganic structures (Waibel et al., 2011). Chitosan also has unique properties, including biocompatibility, biodegradability, bioactivity, and antibacterial activity. Chitosan is also non-toxic, non-immunogenic, and non-carcinogenic (Akncbay et al., 2007, Ikono et al., 2019a,b). In dentistry, chitosan, in the form of an antibacterial gel, has been studied by Akncbay et al. (2007) to treat chronic periodontitis (Suzuki et al., 2013, Bachtiar et al. 2015).

The bacteria around the orthodontic mini-implants are thought to be similar to bacteria in the gingival groove, namely a group of pathogenic bacteria known as the *red-complex*. The *red-complex* bacteria include *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* (Sato et al., 2007). The condition of the tissue around the orthodontic implant's neck resembles the gingival sulcus environment and thus likely supports the growth of anaerobic bacteria, including *red-complex* bacteria (Apel et al., 2009). This condition is thought

to cause an inflammatory and infectious reaction and ultimately causes orthodontic mini-implant failure (Freitas et al., 2012, Bachtiar and Bachtiar, 2017). Here, we hypothesized that chitosan gel would prevent the growth of *P. gingivalis*, *T. forsythia*, and *T. denticola* in the mini-implant during orthodontic treatment.

2. Material and methods

The ethical research committee of Faculty Dentistry Universitas Indonesia approved this study (Number: 31/Ethical Approval/FKG UI/IV/2019). The inclusion criteria were as follows: orthodontic patients with good oral hygiene, using orthodontic mini-implants of the Dual-Top Anchor System (JEIL Medical Corp., Seoul, Korea) for at least two weeks, and the patient had not used mouthwash or other gels in the past one month. The exclusion criteria were as follows: patients with a history of allergy to chlorhexidine or chitosan, systemic disease, and active smoking. The patients had not taken antibiotics in the past month. Patients who met the inclusion criteria were included in this study until the calculated sample size of 30 was reached; they were then divided into three randomized testing groups. The research subjects consisted of 23 female patients (76.67%) and seven male patients (23.33%), aged 16–38 years old. The subjects were administered 0.2% chlorhexidine gluconate gel (Periokin), chitosan 2% gel, or placebo gel containing carboxymethyl cellulose.

2.1. Plaque sampling

Plaque from the orthodontic *peri*-mini-implant area was collected from the area between the immobile gingiva and the transmucosal neck of the orthodontic mini-implant, using absorbent paper point no. 35 (Dentplus No. 35), and then placed into transport medium (1 mL phosphate-buffered saline, Oxoid™, Hampshire, UK). The participants were then given a pack of sterile cotton buds and one tube of gel and asked to use the gel in approximately the size of green beans around the orthodontic mini-implant twice per day in the morning and at night for four days. The subjects were instructed not to rinse, drink, or eat for 30 min after applying the gel. During the four days, the subjects continued cleaning the mini orthodontic implant area and did not use any other antibacterial gels. The participants were instructed to maintain oral hygiene by brushing their teeth twice per day according to the proper and correct way of brushing their teeth and cleaning the area around the orthodontic mini-implants. The plaque samples were stored at -20°C until analysis.

DNA was extracted from each sample, the DNA concentration was calculated, and the Ct values were determined using real-time PCR (Applied Biosystems, StepOne™ Real-Time PCR System). The CFU/mL values for each sample were obtained using a linear equation formula from the standard curve obtained from the Ct values. The standard pure bacterial culture was constructed from DNA prepared from a pooled sample of all patients using primers to detect 16sRNA bacteria. The total bacteria was quantified by comparing the Ct value obtained from real-time PCR analysis of the sample with a standard curve showing the correlation between the Ct value and CFU/mL from the results of standard pure bacterial culture.

The identification and quantification of *P. gingivalis*, *T. forsythia*, and *T. denticola* with real-time qPCR were performed as previously described (Hasriati et al., 2020). The following oligonucleotides were used for qPCR (Bachtiar and Bachtiar, 2018): *P. gingivalis* (forward) 5'-TACCCATCGTCGCCTTGG T-3', (reverse) 5'-CGGACTAAA ACCGCA TAC ACTTG-3'; *T. denticola* (forward) 5'-ATCCTGGCTCAG GAT-3', (reverse) 5'-TACGCATAC CCATCCGCA-3'; *T. forsythia* (forward) 5'-AGAGCAAGCTCTCCCTTACCG T-3', (reverse) 5'-TAAGGGCGGCTTGAAATAATG-3'; total bacteria (forward) 5'-CTCACGACACGA GCTGACGAC-3' (reverse) 5'-TTAAACTCAAAGGAATTGACGG-3'.

2.2. Relative quantification

The proportions of *P. gingivalis*, *T. forsythia*, and *T. denticola* were determined using the $2^{-\Delta\Delta Ct}$ formula as the difference between ΔCt after gel application and before gel application (Enita et al., 2011). The Ct value was the difference between the Ct target and Ct 16s-rRNA (total bacteria). The $2^{-\Delta\Delta Ct}$ value showed the magnitude of the reducing number of bacteria after gel application.

2.3. Data and statistical analyses

To analyze the difference in the total number of bacteria/*P. gingivalis*/*T. denticola*/*T. forsythia* before and after applying chlorhexidine gel, chitosan gel, and control gel, the paired *t*-test was performed if the data distribution was normal. However, if the data distribution was not normal, the Wilcoxon test was performed. The differences between each group, post hoc analysis were performed.

3. Results

The results showed that of the 52 orthodontic mini-implants evaluated, 34 (65.38%) appeared healthy, showed no clinical signs of inflammation, and 18 (34.62%) showed clinical signs of inflammation and redness swelling, and pain of orthodontic implant *peri-mini*. Of the 18 orthodontic mini-implants that showed clinical signs of inflammation, 4 (7.69%) were red and swollen, 6 (11.53%) were red, and there were complaints of pain, and 1 (1.92%) showed redness and swelling, and the patient complained of pain. Seven orthodontic mini-implants (13.46%) showed redness in the surrounding tissue, without swelling, pain, and unsteadiness of the orthodontic mini-implants. No orthodontic mini-implants were found to be shaky.

The results showed a significant difference ($P < 0.05$) between before and after chlorhexidine gel application. There was no significant difference between before and after chitosan gel application. There was a decrease in the total bacteria in the control group, but the decrease was not significant (Fig. 1).

The highest decrease in *P. gingivalis* was observed in the chlorhexidine gel application group, 70.86%, whereas the chitosan gel application group showed a value of 26.59%, with $2^{-\Delta\Delta Ct}$ values of 115, 43.15, and 4.14 in the chlorhexidine, chitosan, and placebo groups, respectively. Placebo gel application had the lowest effect of only 2.55% (Fig. 2). The highest decrease in *T. denticola* was observed in the chlorhexidine gel application group, which was 54.79%, followed by 37.47% after chitosan gel application, whereas the control gel application group showed a minimal effect of only 7.75%. The $2^{-\Delta\Delta Ct}$ values were 20.8, 14.28, and 2.95 in the chlorhexidine, chitosan, and placebo groups, respectively (Fig. 3). The highest decrease in the proportion of *T. forsythia* was observed in the chitosan gel application group, 42.06%, followed by chlorhexidine gel application 30.92%, while the control gel application group showed only a slight effect of about 21.80% (Fig. 4).

There was no significant difference in *P. gingivalis* levels between the gel application group ($P \geq 0.05$). However, gel application significantly reduced the levels of ($P < 0.05$) *T. denticola* and *T. forsythia*.

4. Discussion

This study showed that using a 0.2% chlorhexidine gel effectively reduced the number of bacterial colonization in the area around the orthodontic mini-implant. These results are consistent with previous studies showing that chlorhexidine exhibits good antibacterial activity (Mohammadi, 2008, Nagappan and John, 2012, Bachtiar and Bachtiar, 2018, Jamilian et al., 2019).

The three *red-complex* bacteria were detected in all orthodontic mini-implant areas in this study, both in healthy orthodontic mini-implants and in those showing inflammation signs. Research subjects with good oral hygiene also contained *red-complex* bacteria. The detection of the three *red-complex* bacteria supports the role of *red-complex* bacteria as the cause of infection with orthodontic implant *peri-mini*. In agreement with the results of Park et al. (2012), we observed that cleanliness determines the success of orthodontic mini-implants in the area around the implants.

Chlorhexidine has been used widely for disinfection because of its high antibacterial activity and low toxicity. However, these properties differ at different concentrations and dosage forms. Studies of chlorhexidine were carried out by Jamilian et al. (2019), Restrepo et al. (2015), and Al-Bazi et al. (2016). They showed similar results, with chlorhexidine gel effectively reducing colonies of periodontal bacteria and caries-causing bacteria (Restrepo et al., 2015, Al-Bazi et al., 2016, Jamilian et al., 2019). Furthermore, Al-Bazi et al. (2016) found that long-term use of chlorhexidine gel had no negative impact.

Chitosan reduced the levels of *P. gingivalis*, *T. denticola*, and *T. forsythia* by 26.59%, 37.47%, and 42.06%, respectively. To the best of our knowledge, no studies have examined *T. forsythia* and *T. denticola* in this context, although Akncbay et al. (2007) observed that chitosan has antibacterial effects against *P. gingivalis*. The effectiveness of this antibacterial agent

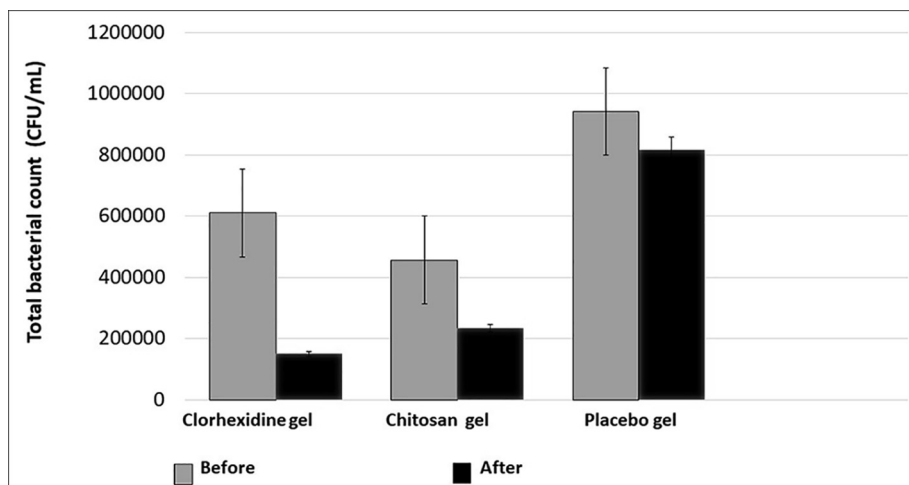


Fig. 1 Total bacterial count before and after gel application. There was a significant decrease after chlorhexidine gel treatment compared to that before treatment. * $P < 0.05$.

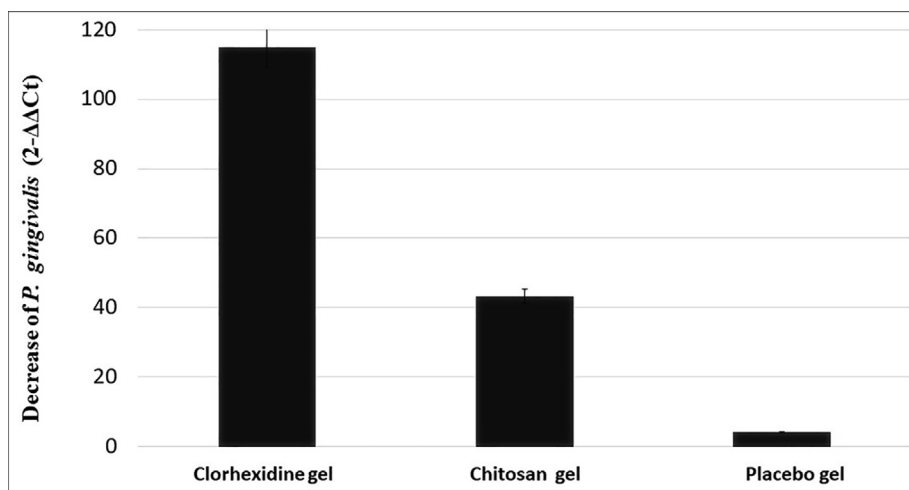


Fig. 2 Decrease in the number of *P. gingivalis* after chlorhexidine gel application compared to that before treatment. * $P > 0.05$.

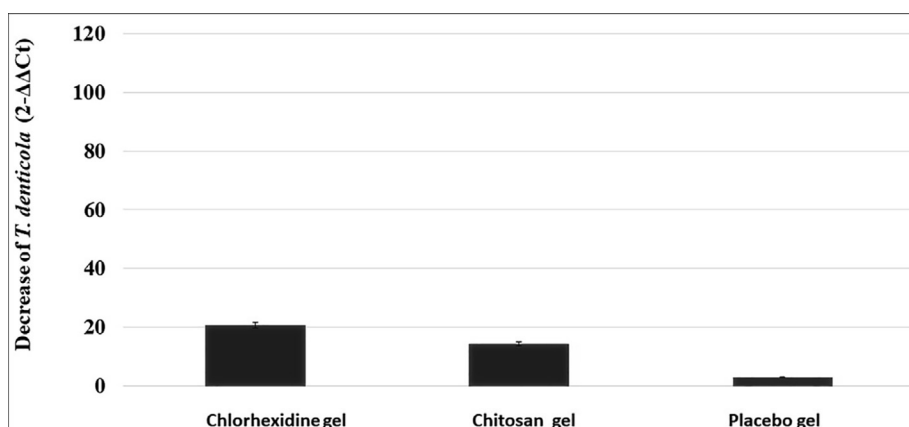


Fig. 3 Decrease in the number of *T. denticola* bacteria after chlorhexidine gel application compared to that before treatment. * $P < 0.05$.

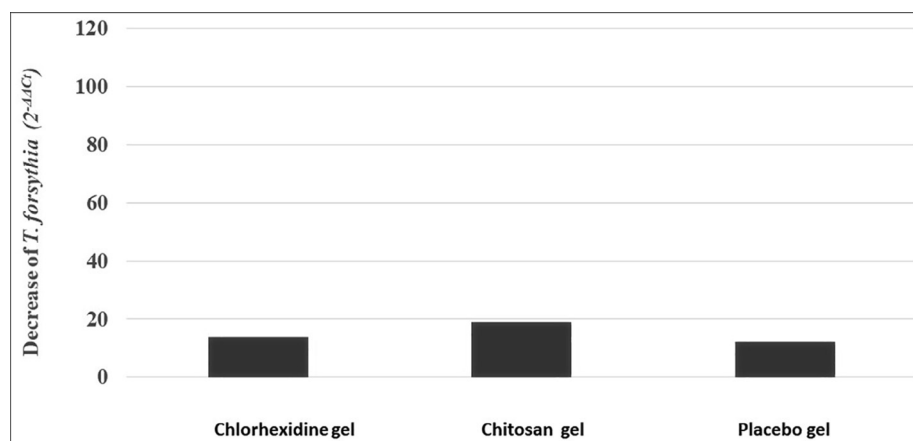


Fig. 4 Decrease in the number of *T. forsythia* bacteria after chlorhexidine gel application compared to that before treatment. *P < 0.05.

requires further analysis of a larger number of samples to optimize the chitosan gel formulation for use as an antibacterial agent.

5. Conclusion

Chitosan gel can reduce total bacterial colonies of *P. gingivalis*, *T. denticola*, and *T. forsythia*, although it is not as effective as chlorhexidine gel.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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