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**ANTIBACTERIAL EFFECT OF TITANIUM DIOXIDE NANOPARTICLES AND  
 CHITOSAN NANOPARTICLES IN ORTHODONTIC ADHESIVE RESIN ON  
 STREPTOCOCCUS MUTANS COLONY**

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**ABSTRACT**

**Back ground:** *Streptococcus mutans* colonization around orthodontic bracket is the common thing that may commence the development of *white spot lesion*. **Objectives:** This research is to analyze antibacterial effect of titanium dioxide nanoparticles and chitosan nanoparticles in orthodontic adhesive resin toward *Streptococcus mutans* colony. **Methods:** This research was conducted at RSKGM University of Indonesia on August 2019. Thirty extracted premolars were randomly allocated into three groups. Bracket was bonded to each specimen using orthodontic adhesive resin (Transbond XT) that was incorporated with titanium dioxide/chitosan nanoparticles. Each sample was submerged in bacterial suspension and was incubated for 24 hours. *Streptococcus mutans* colony around orthodontic bracket was counted with *Total Plate Count* method and then analyzed using statistical analysis. **Results:** There is no difference in *Streptococcus mutans* colony around orthodontic brackets among three treatment groups. **Conclusion:** Antibacterial effect of orthodontic adhesive resins incorporated with titanium dioxide nanoparticles did not differ from orthodontic adhesive resins incorporated with or without chitosan nanoparticles. This is depicted from the number of *Streptococcus mutans* colonies around the orthodontic brackets.

**Keywords:** *Chitosan nanoparticles, Orthodontic adhesive resin, Streptococcus mutans, Titanium dioxide nanoparticles.*

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**INTRODUCTION**

Orthodontic treatment using fixed appliances has become increasingly popular. Its utilization has been common yet increasing the risk for plaque retention and carious lesions around orthodontic brackets and bands, especially in patients with poor oral hygiene.<sup>1</sup> Caries is a dental health problem prompted by multifactorial condition. Four factors have been closely associated with the development of caries, namely host, agent, diet, and time. The lesion may only develop under the interaction of all factors which includes the enamel surface and saliva as the host factor, bacteria as the agent, fermentable carbohydrates as the diet, and time. Concurrently, *Streptococcus mutans* is a bacterial agent commonly found in the oral cavity and significantly contributes to the occurrence of caries. It ferments carbohydrates into lactic acid which triggers the demineralization of tooth enamel. Saliva plays a role as a buffer which can balance the remineralization process, yet the disproportion between demineralization and

remineralization will instigate the formation of dental caries.<sup>2</sup>

Dental caries formation around orthodontic brackets comes forth with the development of early white lesion known as White Spot Lesion (WSL). WSL is a condition frequently encountered during the orthodontic treatment due to bacterial plaque accumulation around the bracket, band and other orthodontic components which shapes facilitate food debris retention. A meta-analysis by Sudararaj et al reported that the incidence of caries during orthodontic treatment reached 45.8% and the prevalence of caries in patient who undergone orthodontic treatment was 68.4%. Factors which may increase the incidence of WSL are young age, poor oral hygiene, male patient, and the duration of treatment.<sup>3</sup>

Plaque control are commonly performed in a conventional approach and focused on the maintenance of oral hygiene which requires mutual support from the patient. One method that may

prevent the demineralization of enamel, in which the discipline and cooperation of the patient are not essential, is the use of orthodontic adhesive resins to fixate orthodontic bracket while withstanding bacterial attachment and biofilm formation.<sup>4</sup>

A material with antibacterial property which may be incorporated into orthodontic adhesive resins is a nanoparticle material.<sup>5</sup> The antibacterial properties of the nanoparticle material are due to its small size and high surface area to volume ratio. This physiochemical property of nanoparticle material facilitates a broad interaction with bacterial membrane so that a wide range of antibacterial effect is achieved.<sup>6</sup>

Nanoparticle materials that can be used in the field of dentistry, especially orthodontic adhesive resins, are comprised of inorganic and organic nanoparticle material.<sup>7-10</sup> One inorganic nanoparticle material that may be incorporated to orthodontic adhesive resins is titanium dioxide due to its transparent color which may not interfere with the color of resin. The addition of titanium dioxide is reported to possess antibacterial property.<sup>7,11</sup> Aside from inorganic nanoparticle, organic particle such as chitosan nanoparticle may also be added in orthodontic adhesive resin. Chitosan nanoparticle is known to be non-toxic, biocompatible, and inexpensive.<sup>12</sup> A study by Sodagar (2016) has proven that antibacterial effectiveness of chitosan nanoparticle in orthodontic adhesive resin is exceptionally good.<sup>8</sup> Nonetheless, Beyth reported that organic nanoparticle is less stable than inorganic nanoparticle.<sup>13</sup> Although it had been proven to possess antibacterial property, antibacterial effectiveness of titanium dioxide and chitosan particle is remained disputable. Moreover, no study has been conducted to compare the effectiveness of organic and inorganic nanoparticle in orthodontic adhesive resin. Hence, the effectiveness of titanium dioxide and chitosan nanoparticle in orthodontic adhesive resin should be investigated.

## LITERATURE REVIEW

### Fixed Orthodontic Appliance and Plaque Accumulation

Fixed orthodontic appliance is reported with an adverse effect of plaque accumulation on the surrounding teeth adjacent to the appliance.<sup>14</sup> This is resulted from the shape of orthodontic component which is prone to food retention. Irregular shape of orthodontic appliance complicates plaque removal and restricts the nature of self-cleaning mechanism.<sup>1</sup>

Bacterial plaque accumulation requires preventive measure to avoid dental caries occurrence around the bracket of orthodontic patient. A preventive approach that may be performed is by improving oral and dental hygiene specifically near the bracket area. This preventive procedure includes mechanical cleaning of toothbrushing, the use of remineralizing agents such as fluoride and casein

phosphopeptides-amorphous calcium phosphates (CPP-ACP), the use of mouthwash and toothpaste containing antibacterial substances, and the addition of antibacterial components to orthodontic adhesive resins.<sup>4,15</sup>

### *Streptococcus mutans*

*Streptococcus mutans* presents naturally as a microbiota of the mouth. These bacteria are mostly found in pits and fissures but the least is found on the buccal surface of teeth. The presence of *S. mutans* in the oral cavity may transform into an opportunistic pathogen that initiates disease and damages to the host. *Streptococcus mutans* is associated with plaque formation and dental caries. These bacteria metabolize sucrose to lactic acid using the enzyme glucose  $C_6H_{12}O_6 \rightarrow 2$  piruvic acid  $\rightarrow 2$  lactic acid + 2 ATP. Acid environment of the mouth resulted from this process forges tooth enamel to be easily demineralized.<sup>16</sup>

### Orthodontic Adhesive

Day-to-day materials to be applied as orthodontic adhesives are glass ionomer cement (GIC), resin modified glass ionomer cement (RMGIC), and composite resin. GIC-based adhesive contains fluoride which may prevent demineralization of teeth, but possess less superior mechanical properties. Composite resin demonstrates good mechanical properties but no demineralization inhibitor was incorporated as the constituent. Therefore, RMGIC was formulated with fluoride content and is expected to provide good mechanical properties such as composite resin. However, the mechanical qualities are not as fine as composite resins, so that the orthodontic adhesive resins remain the most commonly used heretofore.

### Nanoparticle material in orthodontic adhesives

Nanoparticle materials can be incorporated to orthodontic adhesive resins to reduce demineralization caused by orthodontic treatment.<sup>7,11,19,20</sup> The unique antimicrobial properties of nanoparticles are due to the small size particle and associated with the high amount of surface area to volume ratio. These physiochemical properties enhance nanoparticles to have a broad interaction with bacterial membranes, serving them a wide range of antimicrobial effects compared to materials with larger particle.<sup>19</sup>

The smaller the size of the nanoparticles the higher the antibacterial activity. However, antibacterial activity is influenced not only by size but also by its concentration. Several studies have shown that larger nanoparticles are more effective in higher concentration. If the concentration is high, more ions are released to eliminate bacteria.

### Titanium Dioxide Nanoparticle

Predominantly, TiO<sub>2</sub> nanoparticle operates in 2 associating pathways and are off simultaneously by disintegrating cell membrane and producing Reactive Oxygen Species (ROS) known as free radical in the form of oxygen and its derivatives of peroxyde and superoxyde. The first pathway involves membrane disintegration via the electrostatic binding of TiO<sub>2</sub> nanoparticle to bacterial cell wall and membrane which interferes with membrane potential, membrane depolarization and disrupts membrane integration. The loss of membrane integrity causes imbalance in cell transport and respiration that leads to cell death. Further, TiO<sub>2</sub> nanoparticle promotes ROS production. ROS is highly reactive and considered as the most significant factor in nanoparticle cytotoxicity. ROS are normally produced at an equitable level from every cell metabolism. Disproportion in ROS level will generate oxidative stress that later exterminates the components of bacterial cell. Oxidative stress alters membrane permeability and eventually results in membrane damage.<sup>21</sup>

### Chitosan Nanoparticle

Chitosan is a biopolymer polysaccharide obtained from the deacetylation of N-acetylglucosamine that is derived from chitin. Chitin can be discovered in crustacean shell (shrimp, crab, lobster and others), exoskeletons of insects, as well as cell wall of fungi and algae.<sup>22,23</sup>

The formulation of chitosan from chitin is accomplished through several processes that are deproteination, demineralization, decoloration, and deacetylation. Protein in crustacean shell is chemically detached in which the minerals are removed using chloric acid and further advance to its decoloration. Deacetylation or acetyl group removal is preceded to obtain the chitosan.<sup>23</sup>

Chitosan demonstrates several beneficial traits such as non-toxic, biocompatible, biodegradable, and acceptable antibacterial property.<sup>13</sup> Antibacterial mechanism of chitosan has yet been confirmed, yet there are several theories about its working mechanism that are (1) ionic interaction that causes cell wall leakage, (2) inhibition of protein and mRNA synthesis via chitosan infiltration to the nucleus, (3) formation of external barrier to bind metal and suppress essential nutrition for bacterial growth.<sup>24,25</sup>

Factors that influence the antibacterial effectiveness of chitosan are molecular weight and degree of deacetylation. The lower the molecular weight, the higher its effectiveness to reduce bacterial growth. Mobility and ionic interaction are less complex than those in greater molecular weight therefore affecting its binding to bacterial cell membrane. The higher the degree of deacetylation, the higher the effectiveness to lower bacterial growth. Where higher degree of acetyl group is present, the effectiveness of chitosan to reduce bacterial growth is

improved. The more acetyl group are removed, the more free amine group to bind the bacterial membrane.<sup>26</sup>

### MATERIAL AND METHOD

This is an experimental laboratory study which obtained the Ethical Clearance letter from Ethic Commissions of Faculty of Dentistry Universitas Indonesia on July 16th 2019 No. 51/EthicalApproval/VII/2019 and protocol number 050570619. This study was conducted at Oral Biology Laboratory of Faculty of Dentistry Universitas Indonesia.

The double blinded test was deployed using first and second upper premolar extracted prior to the study and fulfilled the inclusion criteria. Inclusion criteria comprises of upper and lower premolar teeth that were extracted for orthodontic treatment. Premolar with restoration or any previous adhesive on the buccal surface, presence of enamel structure anomaly, stain, white spot lesion, caries, cracking, enamel fracture, or malformed were not included.

Specimens of the study were allocated into three treatment groups, that are (1) teeth bonded using orthodontic resin adhesive incorporated with titanium dioxide nanoparticle (n=10), (2) teeth bonded using orthodontic adhesive resin incorporated with chitosan nanoparticle (n=10), and (3) teeth bonded using orthodontic adhesive resin only (n=10). Each specimen was immersed in a test tube with a bacterial solution consisting of sterile sucrose, liquid BHI media and *S. mutans* bacteria stocks for the formation of bacterial colonies. All test tubes were incubated at 37°C for 24 hours. The bacterial specimen was collected through swab technique using a sterile cotton swab around the orthodontic bracket. The cotton bud was inserted into the eppendorf tube containing 1 mL of BHI broth. The specimens taken were inoculated using the pour plate method and the number of *Streptococcus mutans* colonies was calculated using the Total Plate Count (TPC) method. Bacterial growth on agar media can be observed and counted directly without the aid of a microscope. The data were the processed and analyzed statistically using SPSS 21 software.

### RESULTS

The reliability of the measurement results was attained by performing the interobserver and intraobserver test using the Intra Class Correlation (ICC) test. Interobserver and intraobserver test were conducted by measuring the number of bacterial colonies in each treatment group. Based on the ICC test (> 0.80), it can be concluded that there is a very good agreement on the interobserver and intraobserver test. The mean and standard deviation of *Streptococcus mutans* colonies number around the bracket within the treatment groups were obtained by performing univariate statistical tests. The results of

the descriptive analysis for each group are shown in table 1.

**Table 1.** Distribution of *Streptococcus mutans* colonies number in the group of orthodontic adhesive resin incorporated with titanium dioxide nanoparticles, orthodontic adhesive resin incorporated with chitosan nanoparticles, and orthodontic adhesives resin only.

Group	N	Mean±SD	95% Confidence Interval	
			Minimum	Maximum
Adhesive	10	1,51x10 <sup>5</sup> ±1,24x10 <sup>5</sup>	2 x10 <sup>4</sup>	4,5 x10 <sup>5</sup>
Adhesive+TiO <sub>2</sub>	10	2,36x10 <sup>5</sup> ±1,94x10 <sup>5</sup>	2 x10 <sup>4</sup>	6,5 x10 <sup>5</sup>
Adhesive+Chitosan	10	2,09x10 <sup>5</sup> ±1,92x10 <sup>5</sup>	2 x10 <sup>4</sup>	6,0 x10 <sup>5</sup>

To determine the type of hypothesis testing to be used, data normality analysis was carried for each treatment group. Data normality was analyzed using Saphiro Wilk test because the number of specimens was less than 50. All groups (orthodontic adhesive resin incorporated with titanium dioxide nanoparticles group, orthodontic adhesive resin incorporated with chitosan nanoparticles group, and orthodontic adhesive resin only group) demonstrated normal data distribution ( $p \geq 0.05$ ), therefore unpaired T-test was subsequently applied for each pair of treatment groups. The results showed that there was no significant difference in the number of *Streptococcus mutans* colonies around the brackets that were fixated using orthodontic adhesive resin and those bonded using orthodontic adhesive resin incorporated with titanium dioxide nanoparticles ( $p = 0.217$ ). Furthermore, there was no significant difference in the number of *Streptococcus mutans* colonies around the bracket which was bonded using orthodontic adhesive resin and that which was bonded using orthodontic resin adhesives incorporated with chitosan nanoparticles ( $p = 0.185$ ). Based on statistical analysis, it can also be identified that there was no significant difference in the number of *Streptococcus mutans* colonies around the bracket which was bonded using orthodontic adhesive resin incorporated with titanium dioxide nanoparticles and those bonded using orthodontic adhesive resin with chitosan nanoparticles ( $p = 0.972$ ).

## DISCUSSION

This study revealed that there was no difference in the number of *Streptococcus mutans* colonies around the bracket between those bonded using orthodontic adhesive resin incorporated with titanium dioxide nanoparticles, and those bonded using orthodontic adhesive resin only. This study is contrasting with Sodagar et al finding which reported

that the incorporation of 1% titanium dioxide nanoparticle into orthodontic adhesive resin reduced *Streptococcus mutans* colony number. The titanium dioxide nanoparticle in Sodagar et al study is relatively higher than this study and formulated in a disc-shaped mold.<sup>11</sup> This is in accordance with the theory which mentioned that the antibacterial effectiveness of nanoparticles is influenced by the size and concentration used. The smaller the size of the titanium dioxide nanoparticles, the higher the antibacterial effectiveness. The higher the concentration of titanium dioxide nanoparticles, the higher the antibacterial effectiveness.

Another result reported that no difference of *S mutans* colony number was observed between orthodontic adhesive resin incorporated with chitosan nanoparticle group and those in orthodontic adhesive resin only group. This result is opposing Sodagar's (2016) study which affirmed that chitosan nanoparticle in adhesive resin is effective to reduce the number of *Streptococcus mutans* colony.<sup>8</sup>

The effectiveness of chitosan nanoparticles apart from being influenced by their size and concentration, is also influenced by the degree of deacetylation, pH and molecular weight. The higher the pH, the lower the antibacterial activity, and the greater the molecular weight, the lower the antibacterial activity. The chitosan nanoparticles used in this study possessed the same pH and molecular weight as previous studies. Thus, the difference in results that occurred with Moodley's study could be due to the use of nanoparticles with different degrees of deacetylation. In this study, the chitosan nanoparticles used had a deacetylation degree of >85%, while the chitosan nanoparticles used in this study had a deacetylation degree of 75%.<sup>9</sup> According to Younes, the higher the degree of deacetylation, the higher the effectiveness of the bacteria. This happens because more acetyl groups are released and operate

as free amine groups that can bind to bacterial cell wall.<sup>26</sup>

This study also revealed that there was no difference in the number of *Streptococcus mutans* colonies around the bracket between those bonded using orthodontic adhesive resin incorporated with titanium dioxide nanoparticles and those bonded using orthodontic adhesive resin with chitosan nanoparticles. This denotes the antibacterial effectiveness of chitosan nanoparticles that is equal to titanium dioxide (TiO<sub>2</sub>) nanoparticles.

The antibacterial mechanism of a titanium dioxide nanoparticle is remarked by the destruction of cell membrane integrity and the production of Reactive Oxygen Species (ROS). Membrane damage occurs when the positively charged TiO<sub>2</sub> nanoparticles bind electrostatically to the negatively charged bacterial cell walls and membranes, disrupting the membrane potential, membrane depolarization, and losing membrane integrity. In addition, titanium dioxide nanoparticles cause the production of ROS which is highly reactive and causes oxidative stress which damages the components of bacterial cells.

Chitosan nanoparticles have an antibacterial effect because they have a positive charge that binds to the negative charge of the bacterial cell membrane. This electrostatic interaction causes alterations in membrane permeability, resulted to an osmosis imbalance and consequently restricting bacterial growth.<sup>25</sup>

An important stage of bacterial colonization is bacterial attachment on the surface of the teeth and/or orthodontic appliances. Higher surface roughness of orthodontic adhesive resin is reported to facilitate bacterial adhesion. According to Ahn (2010), orthodontic adhesive resin added with nanoparticles has a higher surface roughness compared to those without nanoparticle material. The rough surface of the orthodontic adhesive resin allows bacteria to adhere more easily.<sup>27,28</sup> Therefore, the high amount of *Streptococcus mutans* bacterial colonies may also be precipitated by the roughness of orthodontic adhesive resins containing nanoparticle materials.

The addition of nanoparticles to enhance orthodontic adhesive resin with an antibacterial property causes higher irregularity on the resin surface. The slower growth of bacteria on composites with smoother surface in culture media may explain the reduced bacterial adhesion effect on bacterial number. Titanium dioxide nanoparticles and chitosan nanoparticles incorporated in adhesive resin demonstrates comparable effect of antibacterial property with an orthodontic adhesive resin without the addition of nanoparticle materials on the number of *Streptococcus mutans* bacteria colonies. It is necessary to evaluate the effectiveness of titanium dioxide nanoparticles and chitosan nanoparticles in orthodontic adhesive resins as antibacterial agents with different amounts and concentrations.

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