Original Article

# Green Tea Extract Solutions can Control Bacterial Biofilms Formed on Space Maintainers

*B Gök, Z Kırzıoğlu, M Kıvanç*[1]

Faculty of Dentistry, Department of Pedodontics, Süleyman Demirel University, Isparta, [1]Anadolu University Faculty of Science, Department of Biology, Eskişehir, Turkey

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ABSTRACT

**Background:** Microorganisms in the mouth are protected from negative environmental conditions by forming biofilms; however, the use of anti-plaque agents in children is not preferred due to toxic side effects. Green tea has been reported to have anti-microbial and anti-dental caries properties. **Aims:** The aim of this study was to assess the ability of green tea extract to prevent the formation of biofilm on the teeth of children using space maintainers. **Methods:** Bacteria were isolated from samples obtained from children aged between 8 and 10 years. The micro-titer plate method and Congo red agar were used to assay biofilm formation. Green tea leaves were obtained from Rize, Turkey. Methanol, hexane and distilled water were used for preparing the extracts. The effects of green tea extract and chlorhexidine on biofilm formation were examined using scanning electron microscopy. **Results:** Presence of *S. mutans 3,3, S. anginosus 2.1.b, S. dysgalactie 6.1.4.1, and E. faecium 10.2.* was measured in the biofilm samples. The extracts showed a bacteriostatic effect on the test bacteria, and among the green tea extracts, the methanol extract was found to exhibit the highest efficacy against biofilm formation by *S. mutans 3.3.* **Conclusion:** Green tea extract showed good efficacy in controlling bacterial growth, and is recommended as a better-tasting alternative for daily oral hygiene due to a lack of known side effects.

**Keywords:** *Biofilm, green tea, oral microorganisms, spacemaintainers*

## Introduction

Space maintainers (SM) are used to preserve arch length following the premature loss or elective extraction of teeth. SMs are fixed or removable devices, and can be composed of different materials such as polymethyl methacrylate, cobalt – chrome, nickel-cobalt and glass fiber. As they are in direct contact with the oral microflora, microbial biofilms can grow on their surface.[1]

The oral cavity represents one of the most complex and diverse biofilms in the human body, and several species can be found on the different surfaces of the mouth. The oral biofilm is the source of various oral diseases, including dental caries and gingivitis, and it adheres to areas on tooth surfaces where the forces preventing adhesion are the lowest.[1] Mechanical cleaning methods alone, such as brushing, are not enough to eliminate biofilm, and antimicrobial agents are often used in addition to prevent adhesion of microorganisms in the mouth, reproduction, and biofilm formation.[2,3] Among the various antimicrobial delivery systems, mouthwashes have been found to be one of the safest and most effective vehicles, especially in young children, as they have the ability to deliver therapeutic ingredients to all accessible surfaces in the mouth including the interproximal surfaces.[3,4] Chlorhexidine (Chx) is the most frequently used antimicrobial agent and is accepted as the gold standard, but Chx also has negative effects, which can include staining of teeth and peripheral tissues, oral mucosal erosion, taste disturbances, paresthesia and toxicity. Thus, it is not recommended for pediatric patients.[5]

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***Address for correspondence:*** Dr. B Gök, Faculty of Dentistry, Department of Paediatric Dentistry, Süleyman Demirel University, Isparta, Turkey. E-mail: kazurat40@hotmail.com



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Increases in the prevalence of side effects associated with antibacterial agents have encouraged scientists to research plant-based antimicrobial agents. Patients are seeking products they perceive to be safer, healthier, and without toxic chemical or synthetic ingredients.[5]

In recent years, people have used medicinal and aromatic herbs more frequently due to their antimicrobial features. Herbal antimicrobial agents that are low cost, minimally toxic, and that can be easily found at home, are increasingly desirable. One of the most widely used herbs is green tea. The catechins present in green tea are an important bioactive component with strong antimicrobial properties. It has been shown that mouthwashes made from green tea extracts can be safely used in pediatric patients since they do not cause toxicity.[6] Previous dental studies reveal the use of herbal mouthwashes mainly in adult populations, while there is a lack of research regarding the pediatric population.[7] The aim of this study was to evaluate the effect of green tea extracts and Chx on biofilms formed on space maintainers.

## Methods

### Preparation of tea extracts

Our study used ground and ready to use green tea produced in Turkey's Rize region. The composition of tea was as follows: Moisture content (g/100 g) 3,07 ± 0.09, Ash (g/100 g dw) 6.82 ± 0.50, Crude fiber (g/100 g dw) 10.10 ± 0.51, Phenolic content (g GAE/100 g dw) 22,22 ± 0.80, Catechins (g/100 g dw) 16.01 ± 0.04. For methanol and hexane extracts, 10 g of green tea was mixed with 100 mL of either methanol (99%) or hexane (99%). Next, the mixtures were placed in a shaking incubator at 45°C for 48 hours. The obtained extract was filtered through Whatman no. 1 filter paper (Whatman, CAmlab Ltd., Cambridge, Great Britain) with the help of a vacuum pump. The extract was then placed in a rotary evaporator at 40°C at 60 rpm, to evaporate the methanol or hexane. The obtained material was dissolved in distilled water before use. For extracts prepared with distilled water, 10 g green tea was mixed with 90 mL Brain Heart Infusion liquid medium (BHI) for 15 minutes. The obtained extract was filtered through Whatman no. 1 filter paper (Whatman, Camlab Ltd., Cambridge, Great Britain) with the help of a vacuum pump. The extract was used after sterilization at 121°C for 20 minutes.

### Preparation of space maintainer materials

Polymethyl methacrylate (10 mm × 5 mm × 5 mm) acrylic cubes, 5 mm × 5 mm (cobalt–chrome) bands, 5 mm × 0.7 mm (nickel – cobalt) clasp wires, and glass fiber samples reinforced with composite were prepared from materials used in the making of oral equipment. Metal wire and band samples were autoclave-sterilized at 121°C for 20 minutes. The acrylic samples were sterilized with UV before use (autoclaving would have changed chemical and surface properties).[8]

### Definition of isolates according to their morphological and biochemical properties

Ethical approval for the study was obtained from the Presidency of the Ethics Committee of Süleyman Demirel University Faculty of Medicine (11.02.2013/509). Bacteria were isolated from samples obtained from children aged between 8 and 10 years who use space maintainers, after receiving personal and parental consent. All samples were procured from the Department of Pedodontics, Faculty of Dentistry, Süleyman Demirel University. Samples were cultivated in blood agar, M17, and Mitis Salivarius Agar, and incubated under anaerobic conditions at 35°C for 48 hours. Growing colonies were selected to prepare pure cultures. Gram staining, oxidase, catalase and coagulase tests, and mobility tests were performed on the isolates. For isolates obtained from MS or M17 agar medium with a typical coccus shape, growth tests were performed at 10°C and 45°C, at pH 9.6, and reproduction and sugar fermentation tests at 4% and 6.5 NaCl with API 20 strep.[9] The selected bacteria were identified with automatic RiboPrinter and the use *Eco*RI, according to the manufacturer's instructions.

### Identification of biofilm formation properties of ısolates

Isolates producing biofilm were identified using Congo red agar (CRA)[10] and the microtitration plate method.[11-13] To determine whether isolates would form a biofilm with CRA, they were cultivated in medium prepared by mixing 0.08% Congo red with BHI agar, which includes 1% lactose, and the plates were incubated at 37°C for 18-24 hours in an environment with 10% $CO_2$. At the end of the incubation time, the morphological appearances of growing cultures were examined. Among the isolates, black colonies were identified as positive, and yellow ones, which did not change color, were identified as negative.[10]

To identify biofilms with the microtitration plate method, bacteria were incubated in BHI broth for 24 hours, and fresh cultures of the microorganisms were prepared. Individual BHI broths, each one containing glucose, lactose, fructose, galactose, raffinose, maltose and sucrose at 2%, were prepared. 18 to 24 hour-old fresh cultures of test bacteria were cultivated in wells containing different types of sugar at a 1:40 ratio. Plain broth was used as a negative control. The samples were incubated at 37°C in 10% $CO_2$ for 48 hours. At the end

of the incubation time, the plates were read with the help of a spectrophotometer (Shimadzu, UV-20101PC) at 490 nm. After reading, the nutrient media in the plate was discharged and the plate was washed twice with sterile phosphate buffered saline (PBS). Two hundred microliter of 95% methanol was added to the plate for 15 minutes. Next, the methanol in the plate was discharged, and the plate was left to air dry for 15 minutes. Once the wells were dry, 200 µL of 2% crystal violet dye solution was added for 5 minutes. The dye in the plate was then discharged and the plate was washed twice with PBS. Lastly, 160 µl of 33% (v/v) glacial acetic acid was added to the plate, and the samples were read with the help of a spectrophotometer (Shimadzu, UV-2101PC) at 570 nm. For each bacterium, this study was conducted in triplicate.[11-13]

### Determination of antimicrobial activities of tea extracts

To determine the minimum inhibitory concentrations (MIC) of each tea extracts, a 2-fold serial dilution method was used. Test bacteria ($10^6$ cfu/mL) were cultivated in wells containing Mueller Hinton broth and the green tea extracts, which were diluted by half. Chlorhexidine (250 µg/mL) was used as a comparison substance. The plates were examined for growth after 18-24 hours of incubation at 37°C. Reproduction was checked with tetrazolium chloride (TCC), and the lowest concentration (no reproduction) was recorded as the MIC. All experiments were conducted in triplicate.[14,15]

### Preparation of saliva samples

5 mililiter of non-stimulated saliva were collected in falcon tubes from children with no systemic diseases who had not used antibiotics or probiotic products within two months. Samples were collected at least an hour after a meal and at the same time of the day. The samples were centrifuged at 4°C and 10,000 rpm for 30 minutes. They were soaked in water at 56°C for 30 minutes. The supernatant was drawn into a syringe and filtered with a 0.22 µm filter before use.

### The effect of green tea extracts on biofilm formation on space-maintaining materials

The prepared space maintaining materials were combined with prepared saliva and 2 mL PBS was added. The tubes were incubated by shaking at 37°C for 20 minutes.[15] The material pieces were removed from the saliva and washed with sterile PBS, following which each piece was added to an individual well of a 12-well plate. *S. mutans* 3.3 ($10^6$ cfu/mL) were added to the equipment pieces. Green tea extract was added to each well at the MIC. BHI with 2% sucrose was used as broth. The plates were incubated at 37°C in an environment with 10%$CO_2$ for 48 hours. After the incubation, material pieces in three wells were examined for biofilm formation, while another set was examined for viable bacteria count, another for scanning electron microscopy (SEM), and lastly for check.

Materials prepared to examine the effect of green tea extracts on biofilm formation were incubated for 24 hours, washed with FTS, and then collected in Eppendorf tubes. 1 mL of 90% methanol was added to these pieces and left for 15 minutes. After the methanol was discharged and the materials dried, 1 mL of 2% crystal violet was added for 5 minutes. The samples in Eppendorf tubes were washed, transferred to fresh tubes, and left to dry. Next, 1 ml of 33% (v/v) glacial acetic acid was poured on them. The tubes were agitated and 200 µL transferred to 96-well plates. The study was conducted in triplicate, and the samples were read with the help of a spectrophotometer (Shimadzu, UV-2101PC) at 570 nm.[13] Samples obtained were examined by SEM.

To determine viable colony number from biofilms on space maintaining materials, the material pieces were washed with PBS 3 times and collected into 1.5 mL tubes with 1 mL of physiological saline. They were sonicated for 4 minutes, and serial dilutions were prepared with physiological saline. These dilutions were cultivated in Mitis Salivarius Agar with the drop plate method and incubated at 37°C in 10% $CO_2$ for 48 hours. After incubation, growing colonies were counted.

### SEM analysis

The effects of green tea extract and chlorhexidine on biofilm formation on space maintaining materials were examined using SEM and the method described by Okajima *et al*, with slight modifications.[16] After the biofilm samples on space maintaining materials were prepared, the cells were washed with 0.1 M cacodylate buffer and fixed with 2.5% glutaraldehyde at room temperature for 1-1.5 hours. After fixation, samples were again washed with cacodylate buffer. Post-fixation was carried out with 1% $OsO_4$ for 1 hour. Next, samples were again washed with cacodylate buffer 2-3 times. Dehydration was carried out using an alcohol gradient (30%, 50%, 70%, 90%, and 100%), at 15 minutes for each series and 2 repetitions. After the alcohol gradient, the drying process was immediately carried out in Critical Point Dryer. Samples were then gold-plated and examined using SEM.

### Statistical analyses

The effects of different green tea extracts on biofilm formation of bacteria isolated from the oral environment, as well as readily obtained bacteria, and the effects of green tea extract applied against biofilm formation on

space maintaining materials were analyzed by using Minitab 16.1.0 (2010) packaged software and ANOVA.

## Results

### Definition of ısolates

According to the results of Gram staining and catalase activity, all test bacteria were found to be catalase negative Gram-positive cocci. In the definition prepared in the automatic RiboPrinter system using EcoRI, the isolates were found to be *S. mutans 3,3, S. anginosus 2.1.b, S. dysgalactie 6.1.4.1,* and *E. faecium 10.2* [Figure 1].

### Assessment of bacterial capacity to form biofilm

It was observed that *S. mutans 3,3*, one of the isolated bacteria, formed biofilm at higher levels compared to other bacteria in agar media which contained different types of sugar. Tested bacteria were found to better form biofilm in agar media with glucose and sucrose added [Table 1].

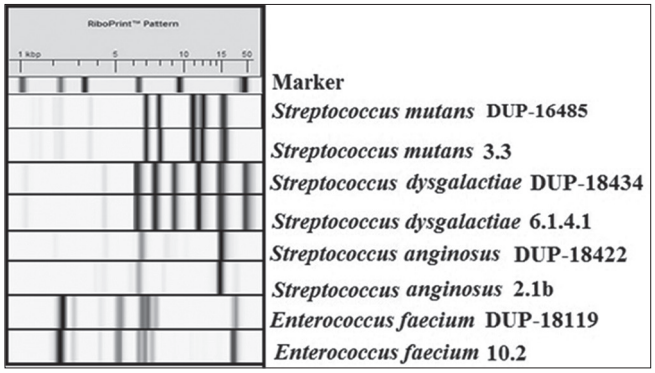


**Figure 1:** The tape profiles of Isolates obtained from the Riboprinter and the standard tape profiles belonging to the 4 species

### Antimicrobial activity of green tea extracts

For *S. mutans 3.3, S. anginosus 2.1.b, S. dysgalactie 6.1.4.1, E. faecium 10.2,* and *S. mutans ATCC 25175*, the MIC values of methanol, hexane and water extracts made with green tea were determined with the micro dilution method [Table 2]. Results showed that none of the tested extracts had a bactericidal effect, instead exhibiting a bacteriostatic effect.

### Effects of green tea extracts on biofilm formation by test bacteria

Examining the effects of green tea extracts on biofilm formation of the bacteria isolated from the oral environment with the help of a spectrophotometer (Shimadzu, UV-2101PC) showed that the differences between groups were statistically meaningful. ($P < 0,05$) [Figure 2]. Results showed that all green tea extracts were effective against biofilm formation. The methanol extract was found to be the most effective against all bacteria tested ($P < 0,05$). The methanol and water extracts were selected for downstream testing of biofilm formation on space maintaining materials.

### Effects of green tea extracts on biofilm formation on space maintaining materials

Only *S. mutans 3.3* was tested on space maintaining materials. The effects of the green tea extracts

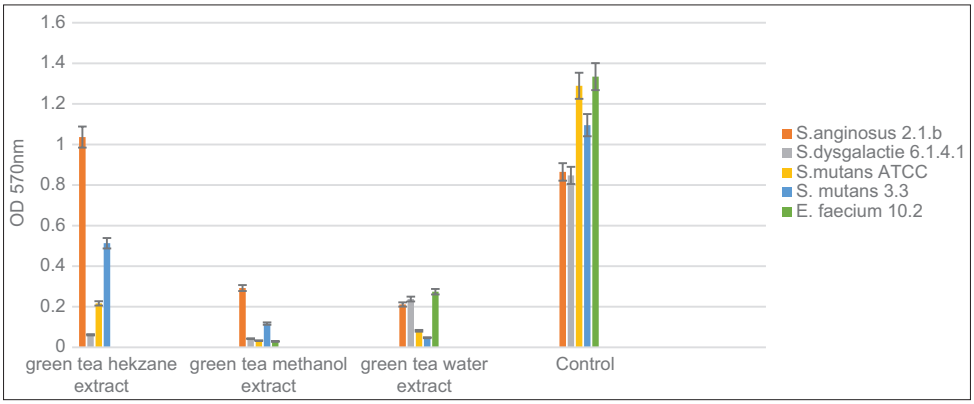


**Figure 2:** Evaluating the effects of green tea and the dry tea leave extracts on the test bacteria regarding the biofilm formation with Spectrophotometer (Shimadzu, UV-2101PC)

**Table 1: Biofilm formation features of bacteria in the media having different sugars**

| Bacterial | Glucose | Fructose | Galactose | Lactose | Maltose | Raffinose | Sucrose |
|---|---|---|---|---|---|---|---|
| *S. dysgalactie* 6.1.4.1 | +++ | +++ | ++ | +++ | +++ | ++ | +++ |
| *S. mutans* 3.3 | +++ | +++ | +++ | +++ | - | + | +++ |
| *E. faecium* 10.2 | + | + | - | + | - | - | ++ |
| *S. anginosus* 2.1b | ++ | ++ | + | + | + | + | ++ |
| *S. mutans* ATTC 25175 | ++ | - | - | - | - | - | ++ |

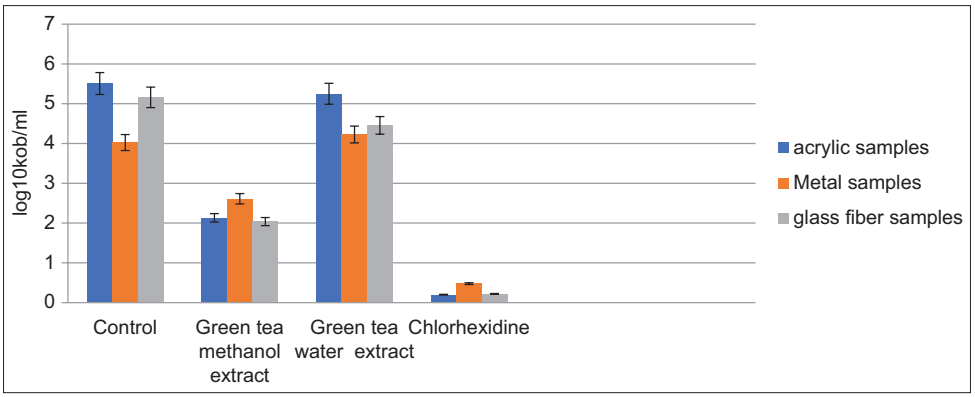


**Figure 3:** Evaluating the effect of tea extracts on the formation of biofilm on the surface of space maintainer apparatus in Log 10 kob/ml

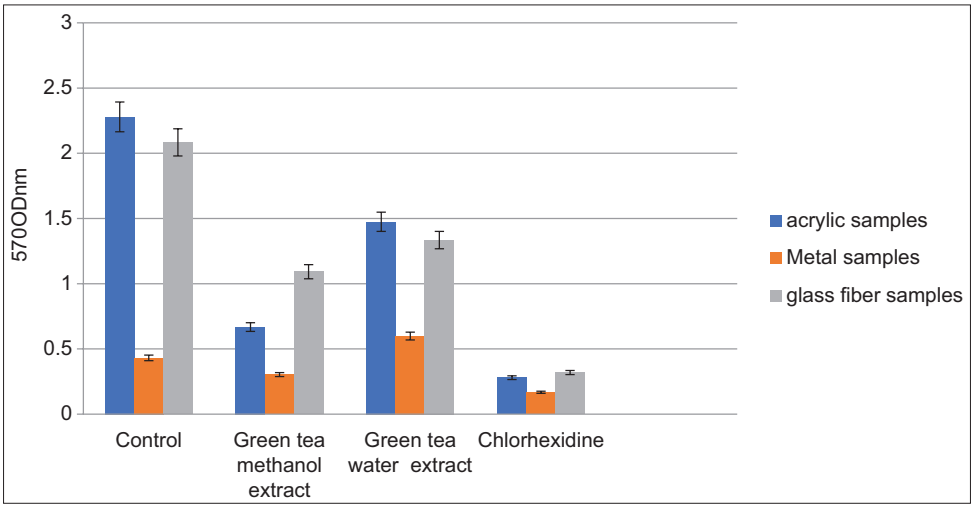


**Figure 4:** Evaluating the effect of tea extracts on the formation of biofilm on the surface of space maintainer apparatus with Spectrophotometer (Shimadzu, UV-2101PC)

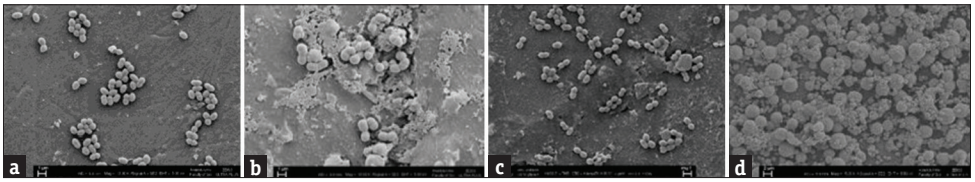


**Image 1:** Acrylic samples SEM images (X10000); (a) Control (b) Green tea methanol extract (c) Green tea water extract (d) Chlorhexidine

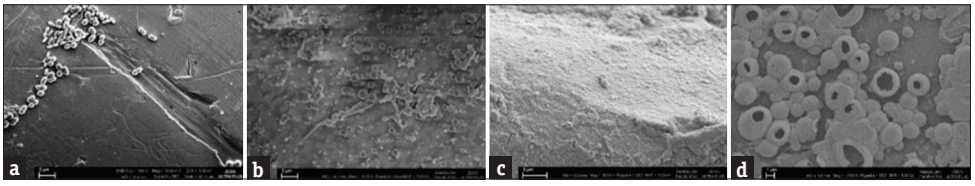


**Image 2:** Metal samples SEM images (X10000); (a) Control (b) Green tea methanol extract (c) Green tea water extract (d) Chlorhexidine

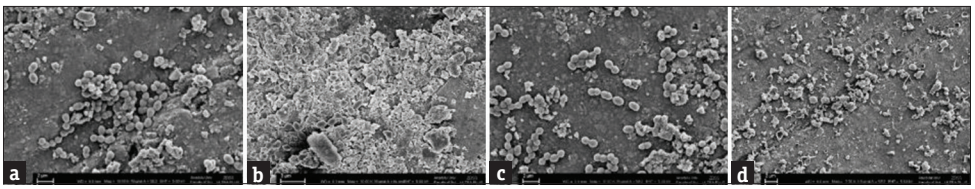


**Image 3:** Fiber samples SEM images (X10000); (a) Control (b) Green tea methanol extract (c) Green tea water extract (d) Chlorhexidine

**Table 2: MIC values of Tea extracts against the bacteria used in the study**

| Bakteria | Green tea | | | |
|---|---|---|---|---|
| | Methanol extract MİC (µl/ml) | Hekzan extract MİC (µl/ml) | Water extract MİC (µl/ml) | Chlorhexidine MİC (µl/ml) |
| *S. dysgalactie* 6.1.4.1 | 9.5 | 11.7 | 625.0 | 0.0011 |
| *S. mutans* 3.3 | 19.0 | 23,4 | 1250 | 0.0011 |
| *E. faecium* 10.2 | 32.0 | 46.4 | 625.0 | 0.0011 |
| *S. anginosus* 2.1b | 9.5 | 23,4 | 1250 | 0.0011 |
| *S. mutans* | 19.0 | 23,4 | 625.0 | 0.0011 |

against biofilm formation by *S. mutans 3.3* on space maintaining materials were assayed by viable bacteria count ($Log_{10}$ kob/ml) and the microtitration plate method (OD 570 nm) as shown in Figures 3 and 4.

Assessing the effects of the green tea extracts on biofilm formation on all space maintaining materials with a spectrophotometer (Shimadzu, UV-2101PC) and the number of bacteria which formed each biofilm revealed meaningful results ($P < 0,05$). Among the green tea extracts, methanol extract was found to be the most effective against biofilm formation by *S. mutans 3.3.*

The rate of biofilm formation was the highest on acrylic materials, and the lowest on metal materials [Figure 4]. On all materials, chlorhexidine inhibited growth of *S. mutans 3.3*., a result also supported by the viable bacteria count and lysed bacteria observed in SEM images [Figures 3 and 4]. Although less effective than chlorhexidine, green tea methanol prevented biofilm formation on all materials. The green tea was the most effective on acrylic and fiber materials, which was also supported by the viable bacteria count. The green tea water extract was the least effective on all materials, which is consistent with the biofilm formation results.

For all groups, our findings were verified with SEM images. These images revealed dense bacterial communities covering the surfaces of space maintaining materials, and extracellular matrix formation which enabled these bacteria to adhere to the surfaces and to each other [Images 1-3].

## Discussion

Space maintainers represent an ideal place for microbial biofilm formation, especially due to their direct contact with the oral microbiota. In addition, space maintainer materials allow the potential for microorganism adhesion and biofilm formation. Chlorhexidine is the most popular mouthwash to control biofilm formation, but displays several side effects, including toxicity, particularly in children. Biofilm formation is a major cause of oral diseases (especially dental caries), and in recent years, studies on developing natural products to prevent biofilm formation suitable for children have gained momentum and popularity.

In this study, the composition of dental biofilms in child patients using space maintainers was identified, and the effects of green tea extracts and 0.12% Chx on biofilm formation by the isolated microorganisms on space maintaining materials were compared.

In our study, the microorganisms *S. mutans 3.3, S. anginosus 2.1.b, S. dysgalactie 6.1.4.1,* and *E. faecium 10.2* were isolated from samples obtained from children who used space maintainers. Similarly, in previous studies *S. sobrinus, S. mitis,* and *S. salivarius* were frequently isolated in addition to *S. mutans*, which is one of the most dominant pathogens.[17,18] Güngor *et al*. observed that *Pediococccus* spp (11.7%), *Lactococcus* spp. (23.5%), *Lactobacillus* spp. (47%), *Weissella confusa* (5.8%), and *S. mutans* (11.7%) were found in the dental biofilms of systemically healthy children.[19] Unlike other studies, here we define microorganisms other than *S. mutans*, which may be due to differences among the study groups in terms of age, sex, dentition period, dietary habits, ethnic properties, systemic and psychological states, and surface properties of the materials of the space maintainer.

In our study, biofilm formations by all isolated bacteria was examined in environments containing different sugars, and the highest level of biofilm formation was observed in environments with sucrose, followed by environments with glucose. Similarly, Ferrazzano *et al*.[20] determined that the highest level of biofilm formation occurred in an environment with sucrose in their research on cariogenic microorganisms, and Cai *et al*., stated that sucrose, among all the fermentable carbohydrates, was the most cariogenic sugar in their research on fermentable carbohydrates,[21] in terms of EPS synthesis of *S. mutans*. Although the isolated microorganisms are different, it can be inferred that the most effective sugar for biofilm formation is sucrose. We observed that among the bacteria compared, *S. mutans 3.3* isolated from the oral environment formed the strongest biofilm, and *S. mutans* ATTC *25175* the weakest, in all agar media regardless of sugar content.

Rosan *et al*. concluded that *S. mutans* was the dominant bacteria and formed the highest level of biofilm in their research on dental plaque formation.[22] Likewise, Lakade *et al*.[17] reported that the most dominant pathogen in dental biofilms was *S. mutans*. Therefore, this organism is frequently used in studies on anti-plaque agents. As mentioned above, our study also identified other microorganisms which had not been defined in previous studies. This allowed the comparison of *S. mutans* to other microorganisms, with the results again showing its importance in oral biofilm formation.

According to our study, none of the extracts exhibited bactericidal effects on the test bacteria, but rather showed bacteriostatic effects. For *S. dysgalactie 6.1.4.1, S. mutans 3.3, E. faecium 10.2, S. angius 2.1.b,* and *S. mutans ATCC 25175* the minimum inhibitory concentration (MIC) values for green tea and water extract were found to be between 625 and 1250 µL/mL. The values for methanol extract were 9.5-32.0 µg/ml, and for hexane extract were 11.7- 46.4 µg/ml. In terms of values reported by other studies, Mankovskaiz *et al*.[23] reported that the MIC values for green tea were between 31.25 and 62.25 mg/ml. Naderi *et al*.[24] stated that the MIC value of the green tea and methanol extract was 150 mg/ml, and the MBC value was 400 mg/ml.

Similar to our study, Simonotti *et al*.[25] and Beighton and Bralisford,[26] in their research on the antimicrobial properties of catechins in green tea, reported that the extracts showed bacteriostatic effects. Contrary to our research, Hamilton and Miller[26] stated that the anticariogenic activities of green tea were bactericidal against *S. mutans* and *S. sobrinus*. Thus, all studies concluded that regardless of the underlying mechanism, green tea had potent antimicrobial effects. These studies also demonstrate that the mechanisms of action may differ based on the origin or exact composition of the green tea extracts.

In our study, the effects of green tea extracts on biofilm formation by the bacteria isolated from the oral environment were identified with the help of a spectrometer (Shimadzu, UV-2101PC). According to the viable bacteria count from the biofilm ($P < 0,05$), we concluded that the methanol and green tea extract was statistically the most effective against all bacteria types. In order of effectiveness, water and green tea extract, and hexane and green tea extract follow the methanol and green tea extract in effect on microorganisms. Similar to our study, Sun and Ho[27] in their study examining different extracts from green tea, state that the methanol extract exerts the strongest antimicrobial effect because it contains more polyphenol. On a related note, Moreno *et al*.[28] asserted that the methanol extract had a stronger antimicrobial effect because it was in correlation with the polyphenols in its structure. Although the origins of the green tea used were different, we also observed in our study that the methanol extract increased the antimicrobial effects of green tea. Collectively, these results confirm the antibiofilm activity of the green tea methanol extract.

The comparison among materials which form space maintainers in terms of biofilm formation by *S. mutans* isolated from oral environment shows that biofilm formation is the highest on acrylic samples, and the lowest on metal. Statistical differences were observed among the groups ($P < 0,05$).

Similarly, studies on acrylic dentures showed that the uneven parts of the surface provide a more favorable environment for the microorganisms compared to other components of the denture.[29,30] In contrast with our study, Kırzıoğlu *et al*.[31] asserted that dental plaque formation was the lowest on fiber space maintainers compared to others, due to their surface properties. Jongsma *et al*.[32] observed that metal materials, especially stainless-steel, had the highest surface tension and therefore the highest plaque retention capacity.[32]

The SEM images of the samples, obtained to examine the relationship between microorganism and surface, showed that the highest and the clearest biofilm formation occurred on acrylic and fiber materials. The results obtained via microtitration plate and viable bacteria count are consistent with the SEM images. While biofilm formation is expected on acrylic materials due to their surface properties, it is interesting that clearer biofilm formation was observed on fiber samples compared to metal ones. These results may be attributed to the shape of the metal samples influencing the stages and results of the study. Similar to our study, Şahin *et al*. observed similar biofilm formation on materials containing acrylic and glass fiber reinforced composite. Although the surfaces of materials used in our research had varying porous structures, SEM analysis showed that there was no significant difference between these materials in terms of adherence of the microorganisms to the surface.[33]

Chx proved to be the most effective compound against biofilm formation by *S. mutans 3.3* on space maintainer materials, with a significant statistical difference between the groups ($P < 0,05$). Excluding Chx, the green tea methanol extract showed the strongest anti-biofilm activity.

Hedge *et al*., in their study comparing the uses of Chx, green tea extract (0.5%), and combination (sodium fluoride and Chx) mouth rinse against

*S. mutans* and *Lactobacillus* colonies in pediatric patients, reported that Chx was more effective compared to the other groups. There was no statistically significant reduction of *S. mutans* or lactobacilli count between the combination mouth rinse and green tea (0.5%) groups.[34] However, green tea has certain advantages over chlorhexidine, including no staining, no lingering after taste, no bacterial resistance, and no known allergies. Moreover, green tea is 5-6 times as cost-effective, easy to prepare, and can be used as a home care product.[35]

Our study and other previous ones concluded that green tea extracts exhibit antimicrobial and antibiofilm activity at a level similar to Chx, despite differing mechanisms. The bactericidal activity of chlorhexidine is presumed to be the result of adsorption of chlorhexidine to extracellular polysaccharides.[36] The cariogenic activity of catechins present in green tea was found to be related to its role in the depletion of a thiol group, which in turn exerted a bactericidal effect and prevented bacterial adherence to teeth, inhibited glucosyltransferase, and human and bacterial amylases.[6,37] One limitation of our study is the lack of available studies on green tea and green tea extract toxicity.

## Conclusion

In children who use space maintainers, green tea methanol extract may be a good alternative to chlorhexidine digluconate mouthwash. However, longitudinal studies under controlled conditions for a longer duration are required to further establish the antiplaque and antibacterial effects of green tea mouthwash in children.

### Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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### Conflicts of interest

There are no conflicts of interest.

## References

1. Kilian M, Chapple IL, Hannig M, Marsh PD, Meuric V, Pedersen AM, *et al*. The oral microbiome – An update for oral healthcare professionals. Br Dent J 2016;221:657-66.
2. Arendorf T, Addy M. Candidal carriage and plaque distribution before, during and after removable orthodontic appliance therapy. J Clin Periodontol 1985;12:360-8.
3. Sundas S, Rao A. Comparative evaluation of effect of chlorhexidine and sodium fluoride mouthwashes on plaque. J Nepal Health Res Counc 2011;13:17-21.
4. Cardoso TR, Carvalho AS, Beletti ME, Napimoga MH, Thedei G Jr. Metabolic activity of Streptococcus mutans biofilms after treatment with different mouthwash formulations. Br J Oral Sci 2011;10:74-8.
5. Saini R, Sharma S, Saini S. Ayurveda and herbs in dental health. Ayu 2011;32:285-6.
6. Hamilton-Miller JM. Anti-cariogenic properties of tea (Camellia sinensis). J Med Microbiol 2001;50:299-302.
7. Prasad KA, John S, Deepika V, Dwijendra KS, Reddy BR, Chincholi S. Anti-Plaque efficacy of herbal and 0.2% chlorhexidine gluconate mouthwash: A comparative study. J İnt Oral Health 2015;7:98-102.
8. Sreenivasan PK, Mattai J, Nabi N, Xu T, Gaffar A. A simple approach to examine early oral microbial biofilm formation and the effects of treatments. Oral Microbiol İmmunol 2004;19:297-302.
9. Dincer E, Kivanc M. Characterization of lactic acid bacteria from Turkish Pastirma. Ann Microbiol 2012;62:1155-63.
10. Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, Melton DM, *et al*. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: A quantitative model for the adherence of staphylococci to medical devices. J Clin Microbiol 1985;22:996-1006.
11. Freeman D, Falkiner FR, Keane C. New method for detecting slime production by coagulase negative staphylococci. J Clin Pathol 1989;42:872-4.
12. Merritt J, Kadouri D, O'Toole G. Growing and analyzing static biofilms. Curr Protoc Microbiol 2005;Chapter 1:Unit 1B. doi: 10.1002/9780471729259.mc01b01s00.
13. Stepanovic S, Vukovic D, Dakic I, Savic B, Svabic-Vlahovic M. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J Microbiol Methods 2000;40:175-9.
14. Cardoso GA, Salgado JM, Cesar Mde C, Donado-Pestana CM. The effects of green tea consumption and resistance training on body composition and resting metabolic rate in overweight or obese women. J Med Food 2013;16:120-7.
15. Song JH, Yang TC, Chang KW, Han SK, Yi HK, Jeon JG. *In vitro* effects of a fraction separated from Polygonum cuspidatum root on the viability, in suspension and biofilms, and biofilm formation of mutans streptococci. J Ethnopharmacol 2007;112:419-25.
16. Okajima Y, Kobayakawa S, Tsuji S, Tochikubo T. Biofilm formation by Staphylococcus epidermidis on intraocular lens material. Invest Ophthalmol Vis Sci 2006;47:2971-5.
17. Lakade LS, Shah P, Shirol D. Comparison of antimicrobial efficacy of chlorhexidine and combination mouth rinse in reducing the Mutans streptococcus count in plaque. J Indian Soc Pedod Prev Dent 2014;32:91-6.
18. Marsh PD, Bradshaw DJ. Dental plaque as a biofilm. J İnd Microbiol 1995;15:169-75.
19. Güngör Ö, Kırzıoğlu Z, Dinçer E, Kıvanç M. Who will win

the race in childrens' oral cavities? Streptococcus mutans or beneficial lactic acid bacteria? Benef Microbes 2013;4:237-45.
20. Ferrazzano GF, Amato I, Ingenito A, De Natale A, Pollio A. Anti-cariogenic effects of polyphenols from plant stimulant beverages (cocoa, coffee, tea). Fitoterapia 2009;80:255-62.
21. Cai J, Jung J, Lee M, Choi H, Jeon J. Sucrose challenges to Streptococcus mutans biofilms and the curve fitting for the biofilm changes. FEMS Microbiol Ecol 2018;94. doi: 10.1093/femsec/fiy091.
22. Rosan B, Lamont RJ. Dental plaque formation. Microb İnfect 2000;2:1599-607.
23. Mankovskaia A, Lévesque CM, Prakki A. Catechin-incorporated dental copolymers inhibit growth of Streptococcus mutans. J Appl Oral Sci 2013;21:203-7.
24. Naderi NJ, Niakan M, Kharazi Fard MJ, Zardi S. Antibacterial activity of Iranian green and black tea on streptococcus mutans: An ın vitro study. J Dent (Tehran, Iran) 2011;8:55-9.
25. Simonetti G, Simonetti N, Villa A. Increased microbicidal activity of green tea (Camellia sinensis) in combination with butylated hydroxyanisole. J Chemoth (Florence, Italy) 2004;16:122-7.
26. Brailsford SR, Shah B, Simons D, Gilbert S, Clark D, Ines I, *et al*. The predominant aciduric microflora of root-caries lesions. J Dent Res 2001;80:1828-33.
27. Sun T, Ho CT. Antioxidant activities of buckwheat extracts. Food Chem 2004;90:743-9.
28. Moreno S, Scheyer T, Romano CS, Vojnov AA. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. Free Radic Res 2006;40:223-31.
29. Auschill TM, Hellwig E, Sculean A, Hein N, Arweiler NB. Impact of the intraoral location on the rate of biofilm growth. Clin Oral İnvest 2004;8:97-101.
30. Peixoto I, Enoki C, Ito I, Matsumoto M, Nelson-Filho P. Evaluation of home disinfection protocols for acrylic baseplates of removable orthodontic appliances: A randomized clinical investigation. Am J Orthod Dentofacial Orthop 2011;140:51-7.
31. Kirzioğlu Z, Ertürk MS. Success of reinforced fiber material space maintainer. J Dent Child (Chicago, Ill) 2004;71:158-62.
32. Jongsma MA, van der Mei HC, Atema-Smit J, Busscher HJ, Ren Y. *In vivo* biofilm formation on stainless steel bonded retainers during different oral health-care regimens. Int J Oral Sci 2015;7:42-8.
33. Sahin C, Ergin A, Ayyildiz S, Cosgun E, Uzun G. Effect of biofilm formation, and biocorrosion on denture base fractures. J Adv Prosthodont 2013;5:140-6.
34. Hegde Raul J, Kamath S. Comparison of the Streptococcus mutans and Lactobacillus colony count changes in saliva following chlorhexidine (0.12%) mouth rinse, combination mouth rinse, and green tea extract (0.5%) mouth rinse in children. J Indian Soc Pedod Prev Dent 2017;35:150-5.
35. Rosy N, Srinivas R, Vikram B, Sandhya S, Chandra Shekar T, Sivakumar P. Effects of green tea on Streptococcus mutans counts – A randomised control trial. J Clin Diagn Res 2014;8:128-30.
36. Hennessey TS. Some antibacterial properties of chlorhexidine. J Periodontal Res Suppl 1973;12:61-7.
37. Si W, Gong J, Tsao R, Kalab M, Yang R, Yin Y. Bioassay-guided purification and identification of antimicrobial components in Chinese green tea extract. J Chromatogr A 2006;1125:204-10.