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Short-term Effect of Mastic Gum on Salivary Concentrations of Cariogenic Bacteria in Orthodontic Patients

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ABSTRACT

Objective: To determine antibacterial activity of chewing mastic gum against the salivary levels of *Streptococcus mutans*, the total number of viable bacteria, and lactobacilli in patients undergoing therapy with fixed orthodontic appliances.

Materials and Methods: In this study, the levels of *S mutans*, lactobacilli, and total cultivated bacteria were measured before and after chewing mastic gum. The antibacterial effects of chewing mastic gum against these microorganisms in saliva were compared with a placebo gum. The counts for orthodontically treated patients were evaluated before chewing gum; just after chewing gum; and after 45, 75, 105, and 135 minutes. Saliva samples taken from the patients were inoculated onto trypticase-yeast-cystine-bacitracin agar for mutans streptococci and onto Rogosa agar for lactobacilli. The agar plates were incubated for 48 hours anaerobically at 37°C. The total number of viable bacteria was then counted.

Results: Just after chewing the mastic gum for 15 minutes, a significant decrease of total bacteria and *S mutans* was observed ($P < .001$). The reduction in lactobacilli was not significant at later first stage ($P > .05$). However, at the end of 135 minutes, there were significantly fewer *S mutans* ($P < .001$), total viable bacteria ($P < .001$), and lactobacilli ($P < .001$) in the oral cavity after chewing mastic gum than after chewing paraffin ($P < .001$). The results show that chewing mastic gum decreased the total viable bacteria, *S mutans*, and lactobacilli in saliva in orthodontically treated patients with fixed appliances.

Conclusion: Chewing mastic gum might be useful in preventing caries lesions.

KEY WORDS: *Streptococcus mutans*, Lactobacilli, Total bacteria, Mastic gum, Saliva, Antibacterial activity.

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INTRODUCTION Return to TOC

It has been reported that metallic brackets induce specific changes in the oral environment, such as a reduction in pH and an increase in plaque accumulation, that further increase the risk of demineralization.[1] Also, fixed orthodontic appliances are considered to be a clinical risk factor in terms of enamel integrity because of plaque accumulation around the bracket base.[2] Thus, increased levels of mutans streptococci and lactobacilli are detected in the oral cavity after bonding orthodontic attachments.[3,4] These microorganisms have been identified as the main pathogens in dental caries, and their presence increases the risk for decalcification.[5]

Studies on the management of oral hygiene in patients undergoing orthodontic treatment have concentrated on the effects of different oral hygiene regimens.[6,7] Chemicals and antibiotics have been used as antibacterial agents against *Streptococcus mutans* to reduce plaque-mediated diseases, including dental caries.[8] However, these chemicals and antibiotics may have adverse effects such as vomiting, diarrhea, and tooth staining.[9,10] Chlorhexidine mouthwashes, as an adjunct to tooth brushing, is effective in the control of gingival inflammation, though prolonged use may cause problems with staining.[11,12] Fluoride mouthwashes significantly reduce the extent of enamel decalcification and gingival inflammation during orthodontic treatment.[13] Thus, further research is needed in natural antimicrobial agents for targeting specific oral pathogens while being safe for the host.

Natural products have recently been investigated as promising agents preventing oral diseases. It has been well documented that medicinal plants confer considerable antibacterial activity against various microorganisms.[14,15] Recently, mastic gum has raised interest in medicine as the public is more aware of the potential hazardous side effects of conventional medications. Mastic gum is the concrete resinous exudate from the stem of the tree *Pistacia lentiscus* Linn, which is cultivated on the Aegean and Mediterranean coasts of Turkey. Mastic gum has been reported to show a broad range of antimicrobial activity[16,17] and to have a bacteriostatic effect in the oral cavity.[17] In our previous study, we demonstrated that mastic gum has an antimicrobial activity against *S mutans* and mutans streptococci in vitro and in vivo.[18] Although the antibacterial activity of mastic chewing gum has already been demonstrated, very few studies have been conducted on bacteria of clinical relevance in dentistry.[17,19]

The aim of this present study was to estimate the antibacterial effect of chewing mastic gum on *S mutans*, lactobacilli, and total viable cariogenic microflora in the mouth of patients undergoing orthodontic treatment.

MATERIALS AND METHODS Return to TOC

Mastic Gum

Mastic gum samples were obtained from the grand bazaar of Istanbul. They were collected from the Fethiye region of Turkey in June 2001.

Patients and Methods

Twenty-five new patients (average age 14.2 ± 1.0 years) with severe malocclusions, allocated for orthodontic treatment at the Department of Orthodontics, Cukurova University, Adana, Turkey, were recruited for the study after informed consent from their families had been obtained. Preadjusted edgewise fixed appliances were planned as the treatment strategy for all patients. The saliva samples from all patients were collected immediately after placing edgewise brackets in the upper and lower dentition. The patients were asked not to eat anything before collection of the saliva samples.

All patients were evaluated in the Department of Periodontology and were selected from those having healthy periodontal condition and good oral hygiene. None received antibiotics or topical antiseptics during the previous 30 days or had systemic diseases that would have altered the amount or composition of the plaque or saliva.

The Ethics Committee of the Faculty of Dentistry, University of Cukurova, approved the study protocols. The experiments were stopped after only 2 weeks. In the first week, paraffin wax was used as a placebo because it has no antibacterial agents and also because it stimulates saliva and helps determine the washing effect of chewing without any agent. In the second week, mastic gum was presented to the patients to chew, and saliva samples were evaluated afterward.

Microbiological Sampling and Processing

We used paraffin wax to determine the washing effect of chewing as a control group and used mastic chewing gum to determine the antibacterial effect of mastic resin. The same patients were used for both control (placebo) and mastic gum experiments in 2 sequential weeks. This study is not a double- or single-blind study.

Before the test, unstimulated saliva was collected by rinsing the mouth with 10 mL of phosphate-buffered saline as a baseline sample. In the first week, paraffin wax was given to the patients as a placebo to chew. In the second week, mastic chewing gum was given to the patients. Saliva samples were collected in sterile test tubes at 30-minute intervals after 15, 45, 75, 105, and 135 minutes from both the paraffin and mastic gum groups. The saliva was kept on ice until tested.

The saliva samples were vigorously mixed on a vortex for 1 minute. Serial dilutions (1:100, 1:10,000, 1: 1,000,000) of the samples were prepared in sterile saline solution. From the serial dilutions, 0.1 mL was transferred and plated on the culture media. Trypticase-yeast-cystine-bacitracin agar supplemented with 18% (wt/vol) sucrose[20] for mutans streptococci and Rogosa agar (Oxoid, Basingstoke, Hampshire, England) for lactobacilli were used as media of choice. Incubation of the samples was performed in an atmosphere of 5% CO_2 at 37°C for 48 hours, then the samples were allowed to develop at room temperature for 24 hours to enhance colony development.

Species identification was based on biochemical tests, including the sugar fermentation tests (acid production from mannitol, sorbitol, melibiose, raffinose, starch, insulin, and dextrin), hydrolysis of arginine and esculin, Voges-Proskauer, and resistance to bacitracin.[21] The fermentation profiles of isolates resembling *S mutans* were also tested by the API 20 Strep System (Bio Merieux, Marcy-l'Etoile, France). *S mutans* ATCC 25175 was used as a reference strain. Fine opaque colonies on Rogosa agar that appeared microscopically as gram-positive rod-shaped cells with a negative catalase reaction were considered to be lactobacilli species.[22] Total viable counts of all cultivable facultative anaerobic bacteria were made on 5% (vol/vol) horse blood agar with tryptone soy agar as a nutrient base. Total viable counts for each bacterial species were made morphologically and were identified with biochemical fermentation tests.[23] Results are expressed as colony forming units (cfu) per milliliter.

Statistical Analysis

Between-group comparisons were made using the Kruskal-Wallis and Mann-Whitney *U*-tests. $P < .05$ was considered significant. The statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS for Windows, version 11.5, Chicago, Ill).

RESULTS Return to TOC

Before the patients in this study chewed mastic gum, the mean total bacteria was 24.4×10^6 cfu/mL, the *S mutans* count was 21×10^4 cfu/mL, and the lactobacilli count was 37×10^3 cfu/mL. The prominent antibacterial effect of mastic gum on total bacteria, *S mutans*, and lactobacilli was determined. This effect was more apparent for total bacteria and *S mutans* than for lactobacilli. In the first 15-minute period, there was a significant reduction in *S mutans* and total bacteria compared with the placebo, whereas this effect was not found for lactobacilli ($P > .05$).

There was a statistically significant reduction in the total bacteria after 15, 45, 75, and 105 minutes ($P < .01$) compared with the placebo gum ($P < .001$), whereas the reduction after 105 and 135 minutes was not significant ($P > .05$). This may be because the mastic gum increases to its maximum effect after 105 minutes (Figure 1).

The reductions of *S mutans* and total bacteria were 21.4% and 28.3% after 15 minutes, 40% and 42.6% after 45 minutes, 58.6% and 52.9% after 75 minutes, 69% and 71.3% after 105 minutes, and 77.6% and 72.9% after 135 minutes (Figure 2).

Also, when the antibacterial effect of mastic gum against lactobacilli was compared with the placebo, no significant reduction was determined after 15 minutes ($P > .05$), whereas a significant reduction was determined in the saliva samples taken after 45 minutes ($P < .001$). Lactobacilli in saliva reduced by 9.6% after 15 minutes, 26.1% after 45 minutes, 61.7% after 75 minutes, 66.8% after 105 minutes, and 74.1% after 135 minutes (Figure 3).

DISCUSSION Return to TOC

The investigations have shown that the oral microflora of the orthodontic patient changes after insertion of a fixed appliance[4] and that the use of orthodontic appliances (fixed or removable) may affect the qualitative and quantitative distribution of the oral microflora.[24,25] Orthodontic appliances are considered to be a clinical risk factor in terms of enamel integrity because of plaque accumulation around the bracket base.[26] Increased levels of mutans streptococci and lactobacilli have been detected in the oral cavity after bonding orthodontic attachments.[3,4]

The results of our study have clearly shown the antibacterial effect of mastic gum on total bacteria, *S mutans*, and lactobacilli. In the in vivo experiments, significant reductions of total bacteria, *S mutans*, and lactobacilli were determined in the saliva of individuals given mastic gum compared with those given paraffin gum.

S mutans and lactobacilli are the organisms that are primarily associated with dental caries.[27] The level of *S mutans* has been shown to be significantly lower in caries-free orthodontic patients.[28] Also, many researchers believe that the insertion of fixed orthodontic appliances, which results in a number of new retention sites, leads to an increase in number of *S mutans* during active orthodontic treatment.[4,28]

When the antibacterial effect of mastic gum against *S mutans* was examined compared with a placebo in each time interval (0, 15, 15–45, 45–75, 75–105, and 105–135 minutes later), a statistically significant reduction was determined. In our previous study, we determined the antibacterial effect of mastic gum on *S mutans* and mutans streptococci both in vitro and in vivo on healthy nontreated individuals. In parallel to our previous study, we determined the antibacterial effect of mastic gum in the mouth of orthodontically treated patients.[18] Also, in a study conducted by Takahashi et al,[17] mastic gum inhibited de novo plaque accumulation compared with a placebo gum, indicating that mastic itself has an anti–plaque-formation activity. The present study's results parallel the study of Takashashi et al.[17]

Recently, antimicrobial therapy with *S mutans* as an indicator organism has been shown to reduce caries activity. Application of disinfectants such as chlorhexidine and iodine directly to the teeth reduce the *S mutans* population in plaque for prolonged periods of time, whereas mouth rinsing with chlorhexidine and oral penicillin therapy does not seem to have a long-lasting effect on the *S mutans* infection.[29–31]

Regardless of which agent is the drug of choice for the treatment of oral diseases, dental scientists are still searching for new therapeutic applications to prevent and treat them. Bitter taste, tooth staining, and temporary alteration of taste can lead to low patient compliance. Toxicity, mucosal ulceration, and development of resistant bacterial strains are additional adverse effects found with several other antibacterial agents. Collectively, these adverse effects of dental medications motivate dentists to use conventional natural therapeutics for the oral cavity ailments.[8–10,17,18]

In the present study, mastic gum isolated from the bark of *P lentiscus* Linn was not found to have most of the above-mentioned adverse effects, and mastic oil was found to have a potent antimicrobial activity in vitro. The antibacterial effect of mastic can be attributed to the presence of alpha-pinene as the active antibacterial ingredient.[16]

In agreement with the studies of Magiatis et al[16] and Takahashi et al,[17] we suggest that regular use of mastic gum may be useful in controlling dental caries because of its antibacterial effect and anti–plaque-formation activity. Because the bacteriostatic effect of mastic was stable under various in vitro conditions simulating those that occur in the mouth, it is potentially useful for development of antibacterial agents applicable for use in mouthwash preparations because of the short treatment time required for the antibacterial effect against lactobacilli.

As a result of our study, the idea of adding an equally or more effective chewing gum product to a patient's mechanical hygiene measures has significant appeal from a convenience and compliance perspective. Routine use of mastic gum may also include situations where mechanical hygiene measures or mouthwashes would be impractical but where a chewable antiplaque agent would be desirable.

Nevertheless, further studies are needed to identify and purify the active ingredients of mastic gum for future use in trials with toothpaste and mouthwash formulas. In addition, longer-term studies will be required to evaluate the advantage of this material, and its bacteriostatic mechanism and specificity against cariogenic bacteria also need to be further researched.

CONCLUSION Return to TOC

- Mastic gum has an antibacterial activity without adverse effects against *S mutans*, lactobacilli, and total bacteria and may be useful for maintaining oral hygiene during orthodontic treatment.

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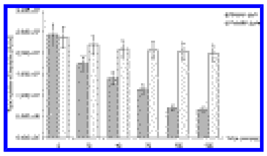

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Figure 1. The antimicrobial activity of mastic gum against the total number of bacteria in the oral cavity

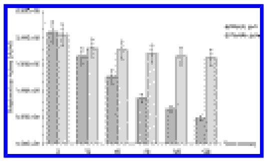

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Figure 2. The antimicrobial activity of mastic gum against *S mutans* in the oral cavity

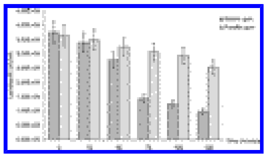

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Figure 3. The antimicrobial activity of mastic gum against lactobacilli in the oral cavity

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