Evaluation of Antimicrobial and Antifungal Effects of Iodoform-Integrating Gutta-Percha

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ABSTRACT

Objective: The antibacterial and antifungal effects of medicated gutta-percha (MGP) over different time periods have not been evaluated. The aim of this study was to evaluate and compare the antimicrobial and antifungal effectiveness of MGP and regular gutta-percha cones over different time periods using the disk diffusion method.

Methods: *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* were spread onto Mueller-Hinton agar, and *Candida albicans* was spread onto Sabouraud agar supplemented with glucose. Same-size MGP cones, conventional gutta-percha cones and disks impregnated with povidone–iodine were placed on the inoculated plates. The dishes were incubated at 37°C aerobically. Growth inhibition zones were inspected and measured at 24, 48 and 72 hours. For each strain, experiments were performed in triplicate. The Kruskal–Wallis test was used to compare *k* independent samples.

Results: The disks impregnated with povidone–iodine inhibited all bacterial strains for up to 72 hours. No inhibition zones were seen around regular gutta-percha cones. MGP inhibited the growth of all bacteria over 24 hours, but in some cases these effects did not continue over longer periods. Specifically, the antimicrobial properties of MGP cones against *E. coli* and *P. aeruginosa* disappeared by 48 and 72 hours. Regardless of time and bacterial strain, MGP was statistically more effective than regular gutta-percha ($p < 0.05$). Povidone–iodine and MGP were effective against *C. albicans* for up to 72 hours, but regular gutta-percha exhibited no antifungal activity.

Conclusion: The antimicrobial and antifungal characteristics of MGP may offer additional advantages over conventional gutta-percha.

MeSH Key Words: bacteria/drug effects; gutta-percha/pharmacology; hydrocarbons, iodated/pharmacology; root canal filling materials

Microorganisms are major etiological agents in pulpal and periapical disease.[1,2] Two of the main goals of endodontic therapy are the elimination of microorganisms from the root canal system and the prevention of subsequent reinfection. Persisting or reinfecting bacteria may induce or sustain apical periodontitis. Yeasts have also been associated with endodontic failures.[1] Therefore, to avoid the growth of these microorganisms, endodontic filling materials should ideally have an antibacterial and antifungal effect.

Gutta-percha has been used to fill root canals for over a century and remains the material of choice for this purpose. Consequently, the antibacterial properties of gutta-percha have attracted the attention of researchers.[3,4]

Evidence of slight antibacterial activity for gutta-percha cones exists. However, the effect

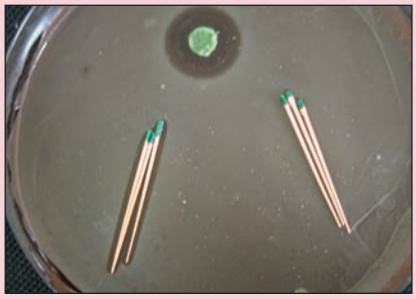

Figure 1: Inhibition of growth of *Enterococcus faecalis* by iodine-integrated gutta-percha.

is too weak for this material to be an effective microbiocide. Moorer and Genet[5] tested the antibacterial properties of gutta-percha cones and found that zinc oxide, which is the major component of gutta-percha, was responsible for some of the antibacterial properties of the cones.

Because the destruction of microbial pathogens is key to endodontic success, Martin and Martin[6] developed medicated gutta-percha (MGP) containing 10% iodoform (triiodomethane) (Lone Star Technologies, Westport, Conn.). The iodoform depot in the MGP core is a biologically active source for inhibiting microbial growth. This new form of gutta-percha has been shown to have antimicrobial effects against *Streptococcus viridans*, *Staphylococcus aureus* and *Bacteroides fragilis*.[6]

The aim of this study was to evaluate and compare the antimicrobial and antifungal effectiveness of MGP and regular gutta-percha cones over different time periods using the disk diffusion method.

Materials and Methods

The following microbial strains were used for the study:

- *Enterococcus faecalis* (ATCC 29212 and ATCC 47077)
- *Pseudomonas aeruginosa* (wild strain)
- *Staphylococcus aureus* (wild strain)
- *Escherichia coli* (wild strain)
- *Candida albicans (ATCC 10231)*

The *E. faecalis* and *C. albicans* strains were obtained from the American Type Culture Collection of Refik Saydam National Hygiene Center in Ankara, Turkey. Saliva samples were obtained from patients at an adult dental clinic, and *P. aeruginosa*, *S. aureus* and *E. coli* were isolated from these samples in the Microbiology Laboratory of the Refik Saydam National Hygiene Center.

All microorganisms except *E. faecalis* were inoculated on agar plates at 37°C. Broth culture suspensions of bacteria and *C. albicans* were adjusted to No. 1 McFarland standard (approximately 3×10^8 cells/mL), and 100-µl aliquots of each microbial suspension were dispersed on the surface of agar plates then transferred to specific fluid growth media before the experiment. *E. faecalis* strains were inoculated at 44.5°C. The microorganisms were grown overnight on the following media: *E. faecalis* (ATCC 29212 and ATCC 47077), *P. aeruginosa*, *S. aureus* and *E. coli* on Mueller-Hinton agar and *C. albicans* on Sabouraud agar with glucose.

Before the experiments, each MGP cone was soaked for 1 hour by complete immersion in 2 mL of sterile water in a test tube to release free iodine.[6]

Identical studies were performed using iodoform-free gutta-percha (Sure-endo Corp., Seoul, Korea). Sterile paper disks, 5 mm in diameter, saturated with 20 µL of 10% povidone–iodine (Isosol, Merkez Laboratory & Medical Tic., Istanbul, Turkey) were used as a control. These control materials were similarly treated.

Iodine-impregnated disks and same-sized standard gutta-percha and MGP cones were aseptically transferred into 3 sections of each previously inoculated plate. No gutta-percha or iodine was applied to the positive control plates.

The plates were incubated aerobically for 24, 48 and 72 hours at 37°C and the antimicrobial effects of MGP, iodoform-free gutta-percha and iodine-impregnated disks were determined by measuring the diameter of zones of inhibition. For each strain, experiments were performed in triplicate, and the average value was determined.

The Kruskal–Wallis test was used to compare k independent samples. Statistical testing was performed with SPSS software (SPSS Inc., Chicago, Ill.).[7]

Results

Uniform growth was evident on all control plates. Samples from triplicate trials yielded consistent results. The mean diameter of zones of inhibition for all tested microorganisms over different time periods are given in **Table 1**.

Povidone–iodine inhibited all strains for up to 72 hours. No inhibition zone was seen around regular gutta-percha cones.

Regardless of time and pathogen strain, MGP was statistically more effective than regular gutta-percha ($p < 0.05$) (**Table 1**). MGP inhibited the growth of all pathogens for 24 hours (**Fig. 1**). The largest mean inhibition zone with MGP occurred with *S. aureus* (mean diameter 10.5 mm), followed in descending order by *C. albicans* (9.3 mm), *E. faecalis* (ATCC 29212) (8.3 mm), *E. faecalis* (ATCC 47077) (7.0 mm), *E. coli* (4.0 mm) and *P. aeruginosa* (3.6 mm). However, not all of these effects continued over time; specifically, MGP did not inhibit the growth of *E. coli* or *P. aeruginosa* after 48 or 72 hours.

Table 1 Nonparametric comparisons of the antimicrobial effects of various materials after 3 time periods

Duration of incubation	Mean diameter of zone of inhibition (mm)			*p* value
	Povidone–iodine	MGP[a]	Regular gutta-percha	
Staphylococcus aureus				
24 h	24.3	10.5	0	0.023
48 h	23.5	9.5	0	0.024
72 h	22.0	8.3	0	0.023
Enterococcus faecalis, ATCC 29212				
24 h	19.7	8.3	0	0.023
48 h	18.0	7.3	0	0.020
72 h	17.6	7.3	0	0.023
Enterococcus faecalis, ATCC 47077				
24 h	16.6	7.0	0	0.023
48 h	15.6	6.1	0	0.023
72 h	14.6	5.5	0	0.023
Escherichia coli				
24 h	12.0	4.0	0	0.021
48 h	11.6	0	0	0.021
72 h	11.6	0	0	0.021
Pseudomonas aeruginosa				
24 h	10.3	3.6	0	0.023
48 h	9.6	0	0	0.021
72 h	9.0	0	0	0.018
Candida albicans, ATCC 10231				
24 h	14.3	9.3	0	0.023
48 h	13.3	8.3	0	0.023
72 h	12.3	7.6	0	0.023

[a]*MGP = medicated gutta-percha*

MGP was moderately effective against *C. albicans* over all time periods, whereas regular gutta-percha exhibited no antifungal activity.

Discussion

Elimination of bacteria from the root canal system is essential for long-term success of endodontic treatment. Additionally, the root filling prevents infection by acting as a barrier to further microbial challenges, entombing any surviving bacteria within the root canal system and stopping periapical tissue fluids from reaching bacterial cells in the root canal.[8]

In the study reported here, MGP and regular gutta-percha cones were tested against 6 strains (5 species) of microorganisms, but an infected root canal can contain more than 5 species of pathogen. In addition, the root canal typically contains both necrotic and viable tissues and tissue fluids, which may reduce the antimicrobial activity of MGP cones. Furthermore, the antibacterial effect of the MGP cones differed according to the strain of microorganism tested. This could be interpreted as selective potency, which might lead to a quantitative or qualitative shift in the composition of the endodontic microflora. It is also conceivable that this variable effectiveness might support a microflora that is resistant to therapy, should retreatment become necessary. Thus, it will be important to confirm that the observed antibacterial effect of MGP cones on various endodontopathogenic microorganisms helps to reduce the overall bacterial population in infected root canals, rather than merely altering the species composition.

In view of the high prevalence of anaerobes and facultative anaerobes in unsuccessful endodontic

therapy,[9] it is important that the antimicrobial activity of root-canal obturation material help to eliminate residual microorganisms unaffected by either chemomechanical preparation or intracanal medication, as well as persistent root canal infection. Therefore, it has been advocated that the root-filling material should have antibacterial and antifungal properties. Antimicrobial chemicals such as iodoform have been added to gutta-percha cones with the intent of retarding the growth of bacteria inside the obturated root canal. It therefore seems important to evaluate the antimicrobial activity of endodontic materials against aerobic and facultative microorganisms.

A facultative anaerobic microorganism, *E. faecalis*, is the major pathogen associated with root canal complications of periapical periodontitis.[10,11] Several authors have reported difficulty in eliminating *Enterococcus* spp. during root canal treatment[9,11]; for example, calcium hydroxide did not completely eliminate *E. faecalis*.[12] Chlorhexidine was effective against *E. faecalis* in vitro,[13] but antibacterial efficacy may differ with different strains of *E. faecalis*, so 2 strains were used in the present study. Silver and others[14] showed that MGP had no inhibitory effect on *E. faecalis* but did inhibit the growth of *Streptococcus sanguis*. Shur and others[15] found that MGP had no efficacy against *E. faecalis, E. coli* or *P. aeruginosa*. Although these findings are compatible with the results reported here for *E. coli* and *P. aeruginosa* after 48 and 72 hours, they differ from the results for *E. faecalis*, against which MGP exhibited mild efficacy. The discrepancies among these studies are probably due to differences in methods and media.

The main antibacterial effect was seen in the first 24 hours, which probably reflects a higher initial rate of release of iodoform from MGP cones, with the subsequent failure of antimicrobial action reflecting decreased release of the iodoform over time. The amount of iodoform released may not suffice to overcome the strong buffer capacity of *E. coli* and *P. aeruginosa*. Therefore, practitioners should not rely on the antimicrobial activity of free iodine released from MGP cones to eliminate microorganisms from root canals.

Attin and others[3] found that regular gutta-percha cones inhibited several bacterial species in vitro. In contrast, in the present study regular gutta-percha cones did not inhibit any of the microorganisms tested. This disparity is probably due to the amount of zinc oxide within the gutta-percha (which differs in different types of gutta-percha) and the use of different bacterial species.

Candida albicans was chosen as a test fungal organism in this study because it has been found in infected root canals.[16,17] MGP had a moderate antifungal effect over all observation periods.

The agar diffusion test used in this study is one of the most frequently used methods for assessment of the antimicrobial activity of endodontic materials.[18–20] It allows direct comparisons of the filling materials against the test microorganisms, indicating which material has the potential to eliminate bacteria in the local microenvironment of the root canal system. However, the size of the inhibition zones does not indicate the absolute antimicrobial efficiency of the material. Solubility and diffusibility in the agar also play important roles.

The results of the present study indicate that the antimicrobial and antifungal characteristics of MGP may offer an advantage over conventional gutta-percha. However, in vitro tests such as the ones performed here can only indicate the potential of some materials to inhibit microbial growth and metabolism in the local microenviroment of the root canal; these materials can then be recommended for further studies in vivo.[21] The present study did not address the clinical performance of MGP.

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