

# Contamination of Gutta-percha Cones Before and During Clinical Use

Hamidreza Razeghi Jahromi<sup>1</sup>, Mostafa Sadeghi<sup>2</sup>, Foad Iranmanesh<sup>3</sup>  , Mahnaz Tashakori<sup>4</sup>

<sup>1</sup>. Student Research Committee, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

<sup>2</sup>. Department of Operative dentistry, Faculty of Dentistry, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

<sup>3</sup>. Department of Endodontic, Faculty of Dentistry, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

<sup>4</sup>. Department of Laboratory Sciences, Rafsanjan university of medical sciences, Rafsanjan, Iran

 **Corresponding author:**

Foad Iranmanesh, Department of Endodontic, Faculty of Dentistry, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

foad.iranmanesh@gmail.com

## Abstract

**Background and Aim:** Decontamination of gutta-percha (GP) cones is recommended before placement in the root canal system. However, the incidence of contamination is still a matter of debate. The present study aimed to evaluate the contamination of GP cones before and during clinical use by general dentists.

**Materials and Methods:** In this in vitro study, 120 GP cones (#20) were examined for incidence of contamination. First, 30 GP packages were opened under aseptic laboratory conditions, and two cones were randomly selected for the laboratory tests. Next, the initially sampled packages were distributed among 30 general dentists and then they were asked to use them clinically for 7 days and then the packages were collected for the microbial tests. The collected cones were placed in tubes containing thioglycolate medium and incubated at 37°C for 21 days. Bacterial growth was detected by presence of turbidity and comparison with the control groups. A sample was collected from the tubes showing turbidity and plated in blood agar and also underwent Gram-staining, followed by colony counting. Data were analyzed using the Chi-square and paired sample t-test ( $\alpha=0.05$ ).

**Results:** None of the 60 samples from initial sampling of packages showed contamination. However, in secondary sampling after clinical use, 8.3% of GP samples were positive for contamination. There was a statistically significant difference in contamination of packages before and after clinical use ( $P= 0.02$ ).

**Conclusion:** Within the limitations of this study, it can be concluded that GP cones can become infected after opening the package and in the process of clinical use.

**Key Words:** Root Canal Therapy; Disinfection; Gutta-Percha; Sterilization

## Article History

Received: 31 June 2021

Accepted: 7 October 2021

**Cite this article as:** Razeghi Jahromi H, Sadeghi M, Iranmanesh F, Tashakori M. Contamination of gutta-percha cones before and during clinical use.

**J Res Dent Maxillofac Sci. 2022; 7(1):41-47. DOI: 10.29252/jrdms.7.1.41**

## Introduction

The success of endodontic therapy mainly depends on removal of microorganisms from the canal and prevention of reinfection of the root canal system. The success rate of endodontic treatment can be enhanced by

decreasing the number of microorganisms without damaging the adjacent vital tissues.[1] Therefore, maintaining the chain of asepsis is extremely important to prevent bacterial contamination of the root canal system. Based on modern-day infection control concepts, the

instruments and materials used during endodontic treatment including gutta-percha (GP) cones must be free from the contaminating microorganisms.[2] Bacteria can enter the root canal via the contaminated endodontic materials. Therefore, root canal disinfection and/or use of materials with antimicrobial activity for root canal obturation, such as GP and various sealers, are among the methods to ensure a germ-free root canal environment.[3]

The commercially available GP cones come in pre-sterilized packages. The scientific literature indicates that GP cones taken from the manufacturer packaging do not need to be sterilized before the first use. Contamination occurs accidentally mainly with continued handling of the packages and through exposure to the physical environment or inappropriate handling by the clinician.[4] Considering these conditions and the importance of preventing cross-contamination during endodontic treatment, it has been recommended to sterilize GP cones before obturation. GP cones cannot be sterilized by conventional autoclaving; therefore, different chemicals have been suggested for this purpose such as zephiran, zephiran chloride, untinted tincture of metaphen, thimerosal, povidone-iodine, alcohol, formaldehyde gas, and glutaraldehyde.[5-7] In recent years, other agents such as chlorhexidine and MTAD have also been suggested.[8] Finally, sodium hypochlorite, the most widely used irrigating solution, has become the material of choice for chairside chemical decontamination of GP.[7]

The most efficient and reliable technique for disinfection of GP cones is immersing them in 5.25% NaOCl for at least 1 minute.[5,6,9-11]

Despite these findings, there have been some controversies regarding the surface alterations of the GP cones due to salt precipitations after using NaOCl as disinfectant, as they can negatively impact the sealing ability when used along with resin-based sealers.[10]

To the best of our knowledge, most clinicians take GP cones directly from their packages, further imposing the risk of contamination by gloves, handling, and/or advertent storage.[12] Contamination of GP cones can occur by handling, aerosols, and also by physical sources during the storage process.[6]

Endodontic studies recommend that the GP cones should be decontaminated before placement in the root canal system.[4,6,13,14] Nevertheless, the incidence of contamination is still a matter of disagreement. Several studies have evaluated the contamination of GP cones before the first usage or the disinfection protocols;[3,4,13,15] however, studies evaluating this occurrence in dental clinics are still lacking. Hence, the objective of this study was to evaluate the contamination of GP cones before and during clinical use by general dentists in 2020.

### Materials and Methods

In this in-vitro study, 30 packages of #20 GP cones (Meta Biomed, Cheongju, South Korea) were used, and two cones were randomly collected from each package immediately after opening (group 1). After sampling, the packages were closed and wrapped in a previously sterilized surgical-grade paper (Empack®, Qom, Iran). The packages were then distributed among 30 dental offices in Rafsanjan city, and the clinicians were asked to use them in the clinic for 7 days. These dental offices were randomly selected from general dentistry offices in Rafsanjan city. This study was conducted during the COVID-19 pandemic. After 7 days of clinical use and storage, the packages were collected from the offices, sealed, and remained sealed until the tests. The entire experiment was conducted under aseptic conditions.

The operator used surgical gloves and sterile instruments. Two cones were selected from each of the 30 GP packages received from the offices (60 samples, group 2) using cotton

pliers and then the cones were immediately placed in test tubes containing 15 mL of thioglycolate medium (Condalab®, Madrid, Spain). The tubes were incubated at 37°C for 21 days in aerobic conditions and assessed daily for the occurrence of turbidity.[13] The tubes that showed visible turbidity were vortexed for 30 seconds. For selective bacteriological identification, 0.1 mL aliquots of these solutions was transferred into tubes, diluted to 10,[3] and streak-cultured on blood agar plates. The agar plates (Condalab®, Madrid, Spain) were incubated at 37°C under aerobic conditions and evaluated after 24 hours. An aliquot from each thioglycolate broth that presented turbidity was subjected to Gram-staining and then the plates were evaluated for colony counting.

One tube containing the culture medium with no sample was used as the negative control of the thioglycolate medium, and one agar plate with no cone was used for the same reason. For the positive control, another tube containing two intentionally contaminated GP cones was used. All tubes and plates were incubated under the same conditions as described above. Demographic information of dental clinicians including age, gender, and work experience, number of root canal treatments performed, disinfection of GP cones, and any change in clinical use of GP during the COVID-19 pandemic were also recorded. The participants were ensured about the confidentiality of their information. Also, the study was approved by the ethics committee of Rafsanjan University of Medical Sciences (IR.RUMS.REC.1399.092).

Descriptive statistics including the mean and standard deviation were used to report the extracted data. Statistical analysis was performed using SPSS version 26. The change in contamination state of the packages after clinical use was analyzed by paired sample t-test. The Spearman's rank correlation coefficient (Spearman's rho) was used to find correlations between environmental

conditions of GP use in the office and presence of contamination. The statistical significance level was set at 5%.

## Results

In the negative control group, no bacterial growth was observed while the positive control group showed bacterial growth. None of the 60 cones initially selected randomly from the packages showed a positive bacterial culture (group 1). In group 2 (60 samples cultured in thioglycolate tubes), 5 tubes showed turbidity after 7 days ( $10^5$  colony forming units counted). GP samples were cultured for 21 days. The colony count did not change from day 7 to day 21. After aerobic culturing, 8.3% of GP cones (5 out of 60) showed positive bacterial culture. Three of the blood agar plates showed bacterial growth confirming the presence of *Staphylococcus epidermidis* in two packages, one plate showed non-hemolytic streptococcus, and one showed Gram-positive bacilli. The results of the paired samples t-test (Figs. 1 and 2) showed that the differences regarding contamination of packages before and after clinical use were statistically significant ( $P= 0.02$ ).

The demographic information of general dentists is presented in Table 1. The Spearman's test showed no significant correlation between age, gender, work experience, decontamination of GP, number of root canal treatments performed, and contamination of GP cones ( $P>0.05$ ).

## Discussion

When using GP cones to fill the root canals, they must be free from pathogenic microorganisms because the goal of endodontic treatment is mainly to eliminate microorganisms from the root canal system and the periapical tissues.[15] The main goal of this study was to investigate the incidence of contamination of GP cones during clinical use. To eliminate the impact of different brands on



Figure 1. GP samples (#20) in thioglycolate medium)

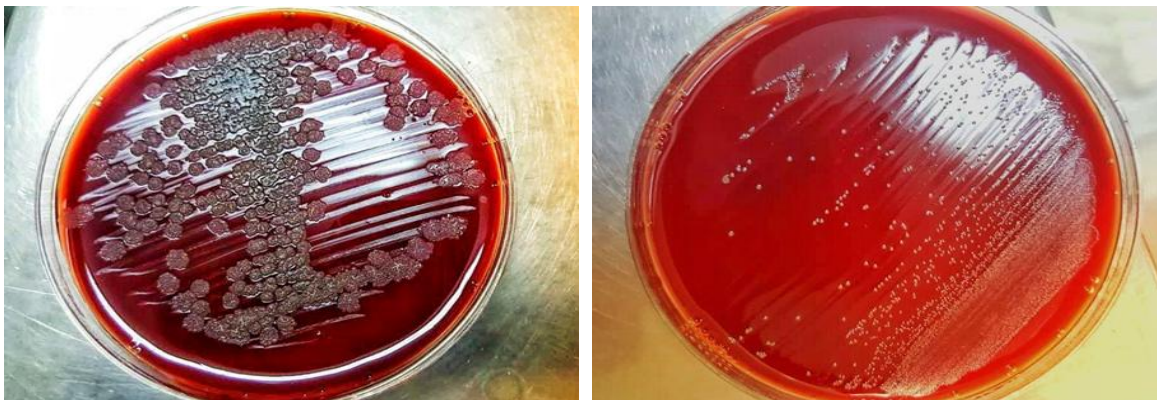


Figure 2. Blood agar plates to ensure bacterial growth

Table 1. Demographic information of general dentists

	Male		Female	
	Mean	Standard deviation	Mean	Standard deviation
Age (years)	39.2	6.1	40.5	5.1
Work experience (years)	10	3.8	9.73	3.3
Number of RCTs*	10.8	4.5	5.4	2.9
Change in method**	Yes		No	
	Male	female	Male	female
Change in method**	4	2	11	13
Disinfection of GP	3	1	10	17

\*: Number of root canal treatments performed in one week

\*\* : Change in method of using GP since the COVID-19 pandemic.

the results, we evaluated only one of the commonly used brands of GP.

The main finding of this study was that the contents of the GP packages were not contaminated but microbial contamination occurred upon clinical use of the packages in some cases. While there was no contamination at the beginning, the clinical use of GP packages led to microbial contamination of some of them. However, no significant correlation existed between the number of root canal treatments performed and the number of microorganisms cultured.

The findings of this study regarding the initial microbial status of the GP cones are in agreement with those of Pang[16] et al, Seabra and Siqueira,[17] and Da Silva et al,[18] who did not find any contamination of GP cones taken from a new package. Meanwhile, our results differed from those of Gomes et al,[14] Kayaoglu et al,[4] and Saeed et al,[3] who found that some of the GP cones taken from the manufacturer's sealed packages harbored microorganisms. A possible explanation for these differences is that our study only tested GP cones manufactured by Meta Biomed Products Co., while the studies cited above that showed contamination tested different brands. There may be differences in manufacturing technology among different manufacturers in terms of aseptic production and packaging. These inconsistencies can be attributed to variations in manufacturing and packaging technology. There are, however, methodological differences among these studies. The latter statement is supported by Gomes et al,[14] who found that the contents of freshly opened GP packages of one brand could be negative while the contents of another brand could be positive for microbial growth. It is noteworthy that even in a particular brand, there may be differences in GP cones in terms of contamination in different manufacturing series.

It was expected that the GP cones would become

contaminated upon opening of the packages and starting their clinical use. As expected, the contamination occurred in 4 of the 30 packages within a total of 7 days. In previous studies, Saeed et al,[3] Kayaoglu et al,[4] and Nacif et al[13] similarly showed that opening and storing the GP packages in the office for normal clinical use may increase the contamination level of the GP cones. The presence of non-oral bacteria such as enteric Gram-negative rods, *Staphylococcus epidermidis*, *Staphylococcus xylosum*, and *Pseudomonas aeruginosa* in infected root canals is highly suggestive of secondary infections.[19,20] In the present study, we found *Staphylococcus epidermidis* after the clinical use of GP cones. Saeed et al,[3] identified *Propionibacterium*, *Staphylococcus*, and *Micrococcus* from the GP packages. Although these bacteria are a part of normal skin flora, they may become opportunistic pathogens and cause nosocomial endodontic infections.[3,21] Niazi et al,[21] found that *Staphylococcus epidermidis* and *Propionibacterium acnes* were the predominant bacteria identified from dentists' gloves and also, they identified huge diversity of bacteria on the gloves. These bacteria, which may be picked up from patient contact or the environment, can be a source of nosocomial endodontic infections.[21] In the present study, we also found *Staphylococcus epidermidis* on GP cones, which can be picked up from the dentists' gloves or patient skin and lead to failure of endodontic treatment. Finding fewer species can be due to the fewer number of samples.

In this experimental study, the thioglycolate broth was used as an enriched medium because it provides the required nutrients for the growth of aerobic and anaerobic microorganisms. This culture not only leads to an increase in the growth rate and count of bacteria in low numbers but also is accurate enough for detecting microorganisms. In tubes where the thioglycolate medium showed

turbidity, contamination was confirmed by Gram-staining. Nacif et al, incubated tubes for 21 days at 37°C and observed turbidity in 3 of the samples after 21 days.[13] In the present study, tubes were incubated for 21 days, and differences between the colony count at 7 and 21 days were not significant ( $P>0.05$ ). However, it seems that 7 days of incubation is enough for any contamination to present as turbidity. Moreover, it would be reasonable to hypothesize that bacterial contamination may increase linearly over time.

According to Panuganti et al,[22] 75% of endodontic postgraduate students did not practice any disinfection protocol for GP cones. Similarly, this study showed that 80% of dentists (24 dentists) did not follow any disinfection protocol for this purpose. Interestingly, three of them (10%) had changed the method of using GP in their office due to the COVID-19 pandemic and so followed the disinfection protocol of GP cones. Therefore, it seems that the basic principle of minimizing the endodontic microbial flora and preventing further contamination has been violated due to not pursuing any of these simple chairside disinfection protocols.

The present findings and those from studies presenting low or no contamination of cones taken directly from their packages, combined with the antimicrobial activity of most root canal sealers, might explain the negligence of cone disinfection before root canal obturation. Nevertheless, there are some arguments in favor of disinfecting cones before use. First, some non-oral bacterial species have been identified in secondary/persistent infections and their source is very likely to be a breach in the aseptic chain (where contaminated GP cones were also included).[23,24] Second, some GP packages, even unpacked, may exhibit contamination.[3,4,13] Third, after opening the package, cones remaining in the package are continuously exposed to the environment during subsequent use and consequently have

a high risk of contamination.

Finally, we had some limitations due to the poor cooperation of some dentists since they were concerned about the confidentiality of their information. However, we assured them in this respect. Another limitation of this study was using only one brand of GP cones; thus, we cannot generalize our results to all brands of GP cones. We used the microbial culture method; however, more species of microorganisms can be detected with more advanced methods. Our findings suggest keeping packages closed when not in use, and using a sterile instrument to take GP cones from the package. Due to the possible contamination of GP cones, disinfection of cones is recommended before use.

## Conclusion

Regarding the limitations of this study, it can be concluded that GP cones can become infected after opening the package and in the process of clinical use.

## Acknowledgements

The present study was conducted in collaboration with Clinical Research Development Unit of Rafsanjan Ali ibn Abi Talib Hospital and hereby the authors express their gratitude and appreciation.

## References

1. Walton RE, Torabinejad M. Principles and practice of endodontics. 5th ed. Philadelphia: Saunders; 2015. Chapter 3, Endodontic Microbiology; p. 37-48.
2. Malmberg L, Björkner AE, Bergenholtz G. Establishment and maintenance of asepsis in endodontics - a review of the literature. *Acta Odontol Scand.* 2016 Aug;74(6):431-5.
3. Saeed M, Koller G, Niazi S, Patel S, Mannocci F, Bruce K, Foschi F. Bacterial Contamination of Endodontic Materials before and after Clinical Storage. *J Endod.* 2017 Nov;43(11): 1852-6.
4. Grossman LI. Endodontic practice. 10th ed. Philadelphia: Lea. & Febiger; 1982:279 p.
5. Kayaoglu G, Gürel M, Omürlü H, Bek ZG, Sadik B.

- Examination of gutta-percha cones for microbial contamination during chemical use. *J Appl Oral Sci.* 2009 May-Jun;17(3):244-7.
6. Senia ES, Marraro RV, Mitchell JL, Lewis AG, Thomas L. Rapid sterilization of gutta-percha cones with 5.25% sodium hypochlorite. *J Endod.* 1975 Apr;1(4):136-40.
7. Monteiro BR, Candoso F, Reis M, Bastos S. Analysis of the performance of contractualization primary health care indicators in the period 2009-2015 in Lisbon and Tagus Valley. *Cien Saude Colet.* 2017 Mar;22(3):807-18.
8. Carvalho CS, Pinto MS, Batista SF, Quelemes PV, Falcão CA, Ferraz MA. Decontamination of Gutta-percha Cones employed in Endodontics. *Acta Odontol Latinoam.* 2020 Apr 1;33(1):45-9.
9. Chandrappa MM, Mundathodu N, Srinivasan R, Nasreen F, Kavitha P, Shetty A. Disinfection of gutta-percha cones using three reagents and their residual effects. *J Conserv Dent.* 2014 Nov. 2014;17(6):571-4.
10. Cardoso CL, Kotaka CR, Redmerski R, Guilhermetti M, Queiroz AF. Rapid decontamination of gutta-percha cones with sodium hypochlorite. *J Endod.* 1999 Jul;25(7):498-501.
11. Vitali FC, Nomura LH, Delai D, Henriques DHN, Alves AMH, da Fonseca Roberti Garcia L, Bortoluzzi EA, Teixeira CS. Disinfection and surface changes of gutta-percha cones after immersion in sodium hypochlorite solution containing surfactant. *Microsc Res Tech.* 2019 Aug;82(8):1290-6.
12. Sailaja PM, Ahmed S, Devi KS, Shiva S. Comparative evaluation of various herbal and synthetic solutions on disinfection of guttapercha: An in vitro study. *Indian J Dent Res.* 2020 May-Jun;31(3):376-81.
13. Makade CS, Shenoi PR, Morey E, Paralikar AV. Evaluation of antimicrobial activity and efficacy of herbal oils and extracts in disinfection of gutta percha cones before obturation. *Restor Dent Endod.* 2017 Nov;42(4):264-72.
14. Nacif MCAM, Marceliano-Alves MFV, Alves FRF. Contamination of gutta-percha cones in clinical use by endodontic specialists and general practitioners. *Rev Fac Odontol Univ Antioq.* 2017 Jun;28(2):327-40.
15. Gomes BP, Vianna ME, Matsumoto CU, Rossi Vde P, Zaia AA, Ferraz CC, Souza Filho FJ. Disinfection of gutta-percha cones with chlorhexidine and sodium hypochlorite. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2005 Oct; 100 (4):512-7.
16. Panuganti V, Vivek V, Jayashankara C, Anilkumar S, Girish S, Nanjundasetty JK. Gutta-percha disinfection: a knowledge, attitude, and practice study among endodontic postgraduate students in India. *Saudi Endod J.* 2016 Sep-Dec;6(3):127-30.
17. Pang NS, Jung IY, Bae KS, Baek SH, Lee WC, Kum KY. Effects of short-term chemical disinfection of gutta-percha cones: identification of affected microbes and alterations in surface texture and physical properties. *J Endod.* 2007 May; 33(5):594-8.
18. Seabra Pereira OL, Siqueira JF Jr. Contamination of gutta-percha and Resilon cones taken directly from the manufacturer. *Clin Oral Investig.* 2010 Jun;14(3):327-30.
19. da Silva EM, Júnior ECS, Marques AAF. Microbiological assessment of contamination of gutta-percha cones used by post-graduation students. *J Health Sci Inst.* 2010 Jun;28(3): 235-6.
20. Sakamoto M, Siqueira JF Jr, Rôças IN, Benno Y. Molecular analysis of the root canal microbiota associated with endodontic treatment failures. *Oral Microbiol Immunol.* 2008 Aug;23(4):275-81.
21. Siqueira JF Jr, Lima KC. *Staphylococcus epidermidis* and *Staphylococcus xylosus* in a secondary root canal infection with persistent symptoms: a case report. *Aust Endod J.* 2002 Aug;28(2):61-3.
22. Niazi SA, Vincer L, Mannocci F. Glove Contamination during Endodontic Treatment Is One of the Sources of Nosocomial Endodontic Propionibacterium acnes Infections. *J Endod.* 2016 Aug;42(8):1202-11.
23. Gatewood RS. Endodontic materials. *Dent Clin North Am.* 2007 Jul;51(3):695-712.
24. Manoil D, Al-Manei K, Belibasakis GN. A Systematic Review of the Root Canal Microbiota Associated with Apical Periodontitis: Lessons from Next-Generation Sequencing. *Proteomics Clin Appl.* 2020 May;14(3):e1900060.
25. Gomes BPFA, Herrera DR. Etiologic role of root canal infection in apical periodontitis and its relationship with clinical symptomatology. *Braz Oral Res.* 2018 Oct 18; 32 (suppl 1):e69.