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ORIGINAL ARTICLE

Contamination of gutta-percha and Resilon cones taken directly from the manufacturer

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Abstract Any substance and material placed in the root canal either temporarily or definitively must be free of microbial contamination. The purpose of the present study was to evaluate the percentage of contamination of Resilon cones, a polycaprolactone-based material, and seven different brands of gutta-percha cones available in the specialized market. Cones were removed from their original manufacturer boxes and immediately transferred to tubes containing thioglycolate broth. Tests were carried out in triplicate. In addition, for quantitative analysis of possible contaminants, cones were taken from their packages, transferred to tubes containing saline solution, agitated, and aliquots of this solution were seeded onto Mueller-Hinton agar plates. No sample showed contamination in any of the tests performed. Despite the absence of detectable contamination before the first use, a rationale for routinely disinfecting cones before placing them into root canals is given.

Keywords Gutta-percha · Resilon · Secondary endodontic infection · Root canal filling · Endodontic treatment

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Introduction

Secondary intraradicular infections are caused by microorganisms that were not present in the primary infection and have penetrated the root canal system during treatment, between appointments, or after the conclusion of the endodontic treatment [21]. Microorganisms can be introduced in the root canal during treatment usually as a result of a breach in the aseptic chain. If microorganisms introduced in the canal manage to adapt to the new environment, surviving, colonizing, and flourishing therein, a secondary infection is established. Environmental contamination of filling materials is a potential source of microorganisms for secondary infections.

Based on the contemporary concepts of infection control, every instrument and material to be placed within root canals should be sterilized. Core filling materials, such as gutta-percha and Resilon cones, are thermolabile and thereby should ideally be provided sterilized by the manufacturer. If not, they should be disinfected chairside through chemical procedures before use in patients. The few studies on this subject have reported that none to up to about 5–8% of gutta-percha cones taken directly from the manufacturer boxes before the first use exhibited some level of contamination [4, 5, 8, 10]. After storage in violated packages, about 19% of cones randomly taken in endodontic clinics were contaminated [12]. Cones from the clinical environmental or intentionally handled with gloves showed contamination with *Staphylococcus* species [5, 12].

Because the majority of manufacturers do not provide information as to the sterility of cones and given the importance of preventing infection or reinfection of the root canal system to achieve an optimum treatment outcome [22], the purpose of the present study was to provide

additional information as to the sterility status of core filling materials available from several different manufacturers.

Material and methods

Gutta-percha and Resilon cones tested in this study are shown in Table 1. Cones of medium size or with equivalent tapers from two different batches of each manufacturer were used (Table 1). The packages remained closed until the tests. The whole experiment was conducted under aseptic conditions inside a laminar flow cabinet (Bio Protector 09, Veco, Campinas, SP, Brazil) previously sterilized using ultraviolet light for 15 min. All testing procedures were performed by a single operator using sterile gloves, mask, and instruments.

For the qualitative analysis, two cones were taken out of each package using cotton pliers and placed directly into a tube containing fluid thioglycolate medium (Fluid Thioglycolate Medium, Merck, Darmstadt, Germany). The procedure was conducted in triplicate for each batch of cones analyzed. Tubes were incubated for 21 days at 37ºC and evaluated daily for occurrence of turbidity, which was indicative of growth.

The dye present in some cones generated a light turbidity that might be taken by growth. In these cases, tubes were vortexed for 30 s and aliquots of 0.1 ml were plated directly onto the surface of Mueller–Hinton agar plates (Difco, Detroit, MI, USA) to check for a bacterial origin. Plates were incubated under aerobic conditions for 3 days at 37ºC. Another aliquot of the broth was subjected to Gram-staining.

For the quantitative analysis, two cones were taken out of their respective original packages, placed directly in a tube containing 1 ml of sterile 0.85% buffered saline solution and vortexed for 1 min. The solution was plated onto the surface of Mueller–Hinton agar plates and incubated under aerobic condition for 3 days at 37ºC.

As negative controls, three tubes containing fluid thioglycolate medium and no sample were incubated for 21 days at 37ºC. The following positive control group, which also served to establish the detection limit of the assay, was included in this study: *Escherichia coli* (ATCC 29213) cells grown in trypticase soy broth (Difco) for 24 h were used to prepare a suspension adjusted to match the turbidity of a 0.5 McFarland $BaSO_4$ standard (approximately 1.5×10^8 cells). This solution was further serially tenfold-diluted until approximately 10^1 colony forming units (CFUs). Gutta-percha cones taken directly from the packages were contaminated in *E. coli* suspensions ranging from 10^1 to 10^8 CFUs for 20 min at 37°C, and after excess medium was dripped off, cones were transferred to fluid thioglycolate broth. This test was carried out in duplicate using one cone per tube at the same incubation conditions described above. The time it took for turbidity to be observed was recorded.

Results

Some tubes showed a slight turbidity when compared to fresh broth. However, they were not suggestive of growth in appearance. To check for a bacterial origin of the turbidity, aliquots of the broth were plated onto Mueller-

Table 1 Materials tested in this study

Cones	Manufacturer	Batch numbers	Constituents[a]
Dentsply (medium)	Dentsply, Petrópolis, RJ, Brazil	214480 and 235160	Gutta-percha, zinc oxide, organic pigments
DiaDent (fine medium)	DiaDent Group International Inc., Burnaby, Canada	010307 and 011006	Gutta-percha, zinc oxide, barium sulfate, coloring agent
Endopoints (medium)	Endopoints, Manacapuru, AM, Brazil	019 and 021	Gutta-percha, zinc oxide, organic pigments
Endopoints-microtipped (medium)	Endopoints, Manacapuru, AM, Brazil	010 and 011	Gutta-percha, zinc oxide, organic pigments
Meta (#25 .04 and .06 taper)	Meta Biomed Co., Cheongju City, Korea	070706.G and 070708.G	Gutta-percha, zinc oxide, barium sulfate, coloring agent
Obtura Spartan (medium)	Precise Dental, Jalisco, México	6626A and 7487E	Gutta-percha, zinc oxide, barium sulfate, coloring agent
Odous (medium)	Odous De Deus Ind. e Com., Belo Horizonte, MG, Brazil	01 and 04	Gutta-percha, zinc oxide, coloring agent
Tanari (medium)	Tanari, Manacapuru, AM, Brazil	007002G and 007005G	Gutta-percha, zinc oxide, organic pigments
Real Seal (#25 .04 and .06 taper)	SybronEndo, Glendora, CA, USA	148118 and 159505	Polyester compound, difunctional methacrylate resin, bioactive glass, radiopaque fillers

[a] According to the manufacturers' information

Hinton agar plates and subjected to gram-staining. These approaches revealed no bacteria, indicating that the change in optical density of the broth was possibly related to dyes present in the composition of the cones. Turbidity suggestive of bacterial growth was verified in only one qualitative test from the brand Odous. Gram-negative rods were visualized after gram-staining of a broth aliquot. However, no growth was seen onto Mueller–Hinton agar plates. As the two other qualitative tests and the quantitative test from the same package all yielded negative results for bacterial growth, this single specimen was discarded from the results and regarded as unintentional contamination during handling. The quantitative test resulted in no growth for all brands of cones used.

Negative control tubes yielded no growth. In the positive controls, cones contaminated with all *E. coli* suspensions (from 10^1 to 10^8 CFUs) showed positive results in thioglycolate broth after 2–3 days, demonstrating the efficacy of the method in detecting even low levels of contamination.

Discussion

Secondary infections may be caused by a breach in the aseptic chain during treatment, which can occur due to the presence of remnants of supragingival biofilm, calculus or caries on the tooth crown; leaking rubber dam; and contamination of endodontic instruments, irrigants, or filling materials [21]. Occurrence of nonoral bacteria, such as enteric gram-negative rods, *Staphylococcus epidermidis, Staphylococcus xylosus*, and *Pseudomonas aeruginosa*, in infected root canals is highly suggestive of secondary infections [7, 13, 14, 20, 23]. Utilization of contaminated root canal filling materials generates a potential for secondary infection to establish and consequently put the treatment outcome at risk. The present study evaluating several gutta-percha brands from different manufacturers and Resilon cones joins others in the literature that found low or no occurrence of contamination for gutta-percha cones when immediately taken from their packages for first use [4, 5, 8, 10]. However, it must be pointed out that cones may become contaminated after the first use or if incorrectly handled [5, 12].

The reason for low levels of or no contamination of the cones as available in their packages may relate to the following: smooth surfaces of the cone, which make bacterial adherence and colonization difficult; lack of proper conditions for bacterial growth, including humidity and nutrient availability; and the content of zinc oxide, which has antibacterial activity and can inhibit colonization [9].

Apparently, the present findings and those from studies presenting low or no contamination of cones taken directly from their packages [4, 5, 8, 10], combined with the antimicrobial activity exhibited by most root canal sealers [1, 2, 11, 17, 19], might be reasons to disregard cone disinfection before filling. Nevertheless, there are some arguments in favor of disinfecting cones before use: (a) some nonoral bacterial species have been detected in secondary/persistent infections and their source is highly likely to be a breach in the aseptic chain (where contaminated cones also fit in) [7, 13, 14, 20, 23]; (b) some cones, even unpacked, may exhibit contamination [4, 5, 8]; and (c) after package violation, cones remaining in the package are continuously exposed to the environment during subsequent use and consequently have a high risk of contamination [5, 12]. Therefore, it seems entirely prudent and justifiable to disinfect cones before use by immersion in NaOCl solution. NaOCl is effective after just 1 min and may not cause significant alterations on gutta-percha cone structure [3, 5, 6, 15, 18, 24]. After disinfection, 96% ethyl alcohol, 70% isopropyl alcohol, or distilled water can be used to remove chloride crystals formed on gutta-percha cones [16].

Conclusions

The present findings demonstrated that Resilon and all gutta-percha cones tested showed no contamination as they are provided from the manufacturer. Even so, given that manufacturers do not guarantee sterility and that cones are usually available in packages that will be opened and handled several times after the first opening, predisposing to environmental contamination, the present findings do not preclude the need to disinfect cones before use. This is in line with the essential role placed by asepsis in the favorable outcome of the endodontic treatment.

Conflicts of interest The authors declare that they have no conflict of interest.

References

1. al-Khatib ZZ, Baum RH, Morse DR, Yesilsoy C, Bhambhani S, Furst ML (1990) The antimicrobial effect of various endodontic sealers. Oral Surg Oral Med Oral Pathol 70:784–790
2. Cobankara FK, Altinoz HC, Ergani O, Kav K, Belli S (2004) In vitro antibacterial activities of root-canal sealers by using two different methods. J Endod 30:57–60
3. da Motta PG, de Figueiredo CB, Maltos SM, Nicoli JR, Ribeiro Sobrinho AP, Maltos KL, Carvalhais HP (2001) Efficacy of chemical sterilization and storage conditions of gutta-percha cones. Int Endod J 34:435–439
4. Gahyva SM, Siqueira JFJ (2001) Avaliação da contaminação de cones de guta-percha disponíveis comercialmente. J Bras Endod (JBE) 4:193–195

5. Gomes BP, Vianna ME, Matsumoto CU, de Paula ESRV, Zaia AA, Ferraz CC, de Souza Filho FJ (2005) Disinfection of gutta-percha cones with chlorhexidine and sodium hypochlorite. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 100:512–517
6. Gomes BP, Berber VB, Montagner F, Sena NT, Zaia AA, Ferraz CC, Souza-Filho FJ (2007) Residual effects and surface alterations in disinfected gutta-percha and Resilon cones. J Endod 33:948–951
7. Haapasalo M, Ranta H, Ranta KT (1983) Facultative gram-negative enteric rods in persistent periapical infections. Acta Odontol Scand 41:19–22
8. Montgomery S (1971) Chemical decontamination of gutta-percha cones with polyvinylpyrrolidone-iodine. Oral Surg Oral Med Oral Pathol 31:258–266
9. Moorer WR, Genet JM (1982) Antibacterial activity of gutta-percha cones attributed to the zinc oxide component. Oral Surg Oral Med Oral Pathol 53:508–517
10. Namazikhah MS, Sullivan DM, Trnavsky GL (2000) Gutta-percha: a look at the need for sterilization. J Calif Dent Assoc 28:427–432
11. Orstavik D (1981) Antibacterial properties of root canal sealers, cements and pastes. Int Endod J 14:125–133
12. Pang NS, Jung IY, Bae KS, Baek SH, Lee WC, Kum KY (2007) Effects of short-term chemical disinfection of gutta-percha cones: identification of affected microbes and alterations in surface texture and physical properties. J Endod 33:594–598
13. Ranta K, Haapasalo M, Ranta H (1988) Monoinfection of root canal with *Pseudomonas aeruginosa*. Endod Dent Traumatol 4:269–272
14. Sakamoto M, Siqueira JF Jr, Rôças IN, Benno Y (2008) Molecular analysis of the root canal microbiota associated with endodontic treatment failures. Oral Microbiol Immunol 23:275–281
15. Senia ES, Marraro RV, Mitchell JL, Lewis AG, Thomas L (1975) Rapid sterilization of gutta-percha cones with 5.25% sodium hypochlorite. J Endod 1:136–140
16. Short RD, Dorn SO, Kuttler S (2003) The crystallization of sodium hypochlorite on gutta-percha cones after the rapid-sterilization technique: an SEM study. J Endod 29:670–673
17. Siqueira JF Jr, Gonçalves RB (1996) Antibacterial activities of root canal sealers against selected anaerobic bacteria. J Endod 22:89–90
18. Siqueira JF Jr, da Silva CH, Cerqueira MD, Lopes HP, de Uzeda M (1998) Effectiveness of four chemical solutions in eliminating *Bacillus subtilis* spores on gutta-percha cones. Endod Dent Traumatol 14:124–126
19. Siqueira JF Jr, Favieri A, Gahyva SM, Moraes SR, Lima KC, Lopes HP (2000) Antimicrobial activity and flow rate of newer and established root canal sealers. J Endod 26:274–277
20. Siqueira JF Jr, Lima KC (2002) *Staphylococcus epidermidis* and *Staphylococcus xylosus* in a secondary root canal infection with persistent symptoms: a case report. Aust Endod J 28:61–63
21. Siqueira JF Jr, Rôças IN (2009) Endodontic microbiology. In: Torabinejad M, Walton RE (eds) Endodontics. Principles and practice, 4th edn. Saunders/Elsevier, St. Louis, pp 38–48
22. Siqueira JF Jr, Rocas IN (2008) Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod 34(1291–1301):e1293
23. Siren EK, Haapasalo MP, Ranta K, Salmi P, Kerosuo EN (1997) Microbiological findings and clinical treatment procedures in endodontic cases selected for microbiological investigation. Int Endod J 30:91–95
24. Valois CR, Silva LP, Azevedo RB (2005) Structural effects of sodium hypochlorite solutions on gutta-percha cones: atomic force microscopy study. J Endod 31:749–751