# The antimicrobial efficacy of 'MGP' gutta-percha *in vitro*

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## Abstract

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**Aim** To determine whether 'MGP' gutta-percha (Westport, CT, USA), a commercially available gutta-percha containing iodoform, inhibits the growth of potential endodontic pathogens.

**Methodology** Inocula of *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus sanguis*, *Fusobacterium nucleatum* and *Actinomyces odontolyticus* were spread onto the surface of agar plates. 'MGP' gutta-percha cones presoaked in sterile water were transferred to the inoculated agar and incubated at 37 °C aerobically or anaerobically as required for optimal growth. Identical studies were performed using iodoform-free gutta-percha and sterile paper disks saturated with 10% povidone–iodine. Following incubation, zones of inhibition around the 'MGP' gutta-percha, iodoform-free gutta-percha and disks were evaluated.

**Results** Povidone–iodine inhibited all the strains. Iodoform-free gutta-percha inhibited *S. sanguis* and *A. odontolyticus*. 'MGP' gutta-percha inhibited *S. aureus*, *S. sanguis*, *A. odontolyticus* and *F. nucleatum*. Neither iodoform-free gutta-percha nor 'MGP' gutta-percha inhibited growth of *E. faecalis*, *E. coli* or *P. aeruginosa*.

**Conclusions** Compared to iodoform-free gutta-percha, iodoform-containing 'MGP' gutta-percha had an inhibitory effect *in vitro* on *S. aureus* and *F. nucleatum*, but not on *E. faecalis*, *E. coli* or *P. aeruginosa*.

**Keywords:** antimicrobial, gutta-percha, *in vitro*, iodoform, 'MGP' gutta-percha.

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## Introduction

Bacteria are crucial to the development of pulpal and periradicular disease (Kakehashi *et al.* 1966, Sundqvist 1976). The bacterial flora associated with infected pulps is polymicrobial and predominantly anaerobic (Sundqvist 1976, Siqueira *et al.* 2000, Rolph *et al.* 2001), whereas the flora in teeth with persisting periapical lesions contains fewer species and proportionally less anaerobes (Sundqvist *et al.* 1998). Whilst *Enterococci* usually make up a small proportion of the initial flora in the untreated root canal (Sundqvist 1992, Sjögren *et al.* 1997, Siqueira *et al.* 2002), they are the species most commonly recovered from root canals of teeth with failed root treatment (Molander *et al.* 1998, Sundqvist *et al.* 1998, Hancock *et al.* 2001) and have been implicated in persistent root canal infections (Haapasalo *et al.* 1983, Byström & Sundqvist 1985). Ideally, all bacteria should be eradicated prior to obturation; however, bacteria may persist in the root canal system despite debridement and disinfection (Haapasalo & Örstavik 1987, Nair *et al.* 1990, Sjögren *et al.* 1997, Sundqvist *et al.* 1998). Recent data indicate that 5% iodine potassium iodide might reduce the frequency of persisting *Enterococci* in the root canal system (Molander *et al.* 1999, Peciuliene *et al.* 2001).

Grossman (1940) advocated that an ideal root canal filling material should be bacteriostatic. Whilst the zinc oxide in gutta-percha cones might impart antimicrobial activity (Moorer & Genet 1982a), until recently, contemporary gutta-percha cones were neither intentionally antiseptic nor bacteriostatic. Recently, iodoform has been included in gutta-percha cones (Lone Star Technologies,

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Westport, CT, USA); this product is marketed as 'MGP' gutta-percha and has US Food and Drug Administration (FDA) approval. The developers state that 'the iodoform in the MGP cones remains inert until it comes in contact with tissue fluids that activate the free iodine' (Martin & Martin 1999). *In vitro* investigations by the developers showed 'MGP' gutta-percha to have antimicrobial activity against *Streptococcus viridans*, *Staphylococcus aureus* and *Bacteroides fragilis* (Martin & Martin 1999).

To date, minimal data are available on iodoform-containing 'MGP' gutta-percha points. By observing zones of inhibition, 'MGP' gutta-percha had antimicrobial activity against *Streptococcus sanguis*, but not against *Enterococcus faecalis* (Silver *et al.* 2000). Preliminary reports (abstracts only) indicated that 'MGP' gutta-percha was ineffective against *E. faecalis* in an *in vitro* leakage study (Bruchmiller *et al.* 2000), and had no antimicrobial activity against bacteria from human oral plaque samples (Dryden *et al.* 2000). The aims of the present *in vitro* investigation were to independently assess the developers' findings and to determine, using the developers' methodology, whether gutta-percha containing 10% iodoform ('MGP' gutta-percha) inhibited the growth of aerobic and anaerobic bacterial species associated with endodontic infections.

## Materials and methods

### Microorganisms

The eight bacterial strains selected for study are listed in Table 1. *S. aureus* was obtained from the American Type Culture Collection (Manassas, VA, USA). All other bacteria were recovered from strain stocks stored at −80 °C in the University of Michigan School of Dentistry, Ann Arbor, MI, USA.

### Antimicrobial experiments

*E. faecalis*, *S. aureus*, *P. aeruginosa* and *E. coli* were grown in 5 mL of Todd Hewitt Broth (THB; Difco, Detroit, MI, USA) and incubated for 24 h at 37 °C aerobically. After 24 h, each sample was diluted 1 : 10 in THB, and 50 μL were spread onto the surface of Trypticase Soy Agar (TSA; Difco, Detroit, MI, USA) using a sterile glass spreader.

The developers stated that the iodoform within the 'MGP' gutta-percha cones remains inert until it comes in contact with tissue fluids, which then activate the free iodine; they used a 'leakage agent' in their study to release the free iodine (Martin & Martin 1999). Adopting the developers' methods, the 'MGP' gutta-percha cones were 'soaked' by complete immersion in 2 mL of sterile water in a test tube for 1 h (Martin & Martin 1999). Control gutta-percha points without iodoform (Hygenic Corp., Akron, OH, USA) were similarly treated. Following soaking, cones were transferred to inoculated agar plates as described below. For each agar plate, a set of five cones were individually soaked.

Four TSA plates were used for each strain and treated as follows:

- Plate 1: Five 'MGP' gutta-percha points (size 30) were aseptically transferred to the centre of each previously inoculated plate.
- Plate 2: Five gutta-percha points (size 30) without iodoform were aseptically transferred to the centre of each previously inoculated plate.
- Plate 3: A sterile paper disk, 7 mm in diameter, saturated with 20 μL of 10% povidone–iodine (Betadine, Purdue Frederick Co., Norwalk, CT, USA) was aseptically transferred to the centre of each previously inoculated plate.
- Plate 4: This plate received no other treatment and served as a positive control for growth of the microorganisms.

All plates were incubated for 24 h at 37 °C aerobically. For each strain, experiments were performed in triplicate.

For experiments using strains grown under anaerobic conditions, the procedures were identical to the above with the following variations:

**Table 1** Antibacterial effects of 'MGP' gutta-percha, regular gutta-percha and povidone–iodine disks

| Bacterial strains | 'MGP' gutta-percha | Regular gutta-percha | Povidone–iodine |
|---|---|---|---|
| *E. faecalis* (OGI) | − | − | + |
| *E. faecalis* (ATCC 47077) | − | − | + |
| *S. aureus* (ATCC 6538) | + | − | + |
| *P. aeruginosa* (UME) | − | − | + |
| *E. coli* (SM10λpir) | − | − | + |
| *S. sanguis* (ATCC 10556) | + | + | + |
| *F. nucleatum* (ATCC 25586) | + | − | + |
| *A. odontolyticus* (ATCC 17982) | + | + | + |

+, antimicrobial; −, not antimicrobial.

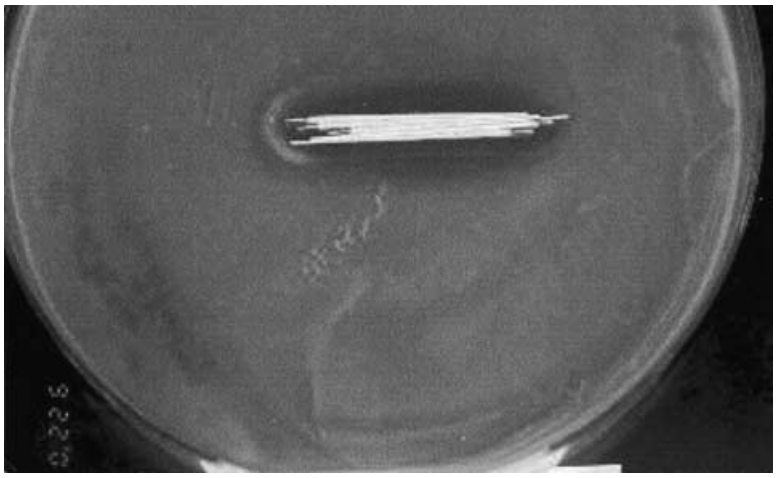

**Figure 1** Inhibition of growth by 'MGP' gutta-percha.

1 Microorganisms were grown in Schadler broth (Difco, Detroit, MI, USA).
2 *S. sanguis* was incubated for 24 h and *A. odontolyticus* and *F. nucleatum* were incubated for 48 h at 37 °C in an anaerobic culture chamber.
3 Anaerobes were spread on blood agar plates (Anaerobe Systems, Morgan Hills, CA, USA).
4 Plates were incubated for 24–48 h in an anaerobic culture chamber at 37 °C.

After incubation, all the plates were visually inspected by two examiners for evidence of growth on the control plates and inhibition of bacterial growth on the other plates. Absence of microbial growth immediately adjacent to the 'MGP' gutta-percha, regular gutta-percha and iodine disks indicated antimicrobial activity.

## Results

Uniform growth was evident on all control plates. Results for all other plates are presented in Table 1. Samples from triplicate trials yielded consistent results.

Povidone–iodine inhibited all strains of bacteria tested. Gutta-percha without iodoform inhibited *S. sanguis* and *A. odontolyticus* only. 'MGP' gutta-percha inhibited the growth of *S. sanguis*, *A. odontolyticus*, *F. nucleatum* and *S. aureus* (Fig. 1), but not *E. faecalis*, *P. aeruginosa* and *E. coli* (Fig. 2).

## Discussion

In the absence of a coronal seal, 50% of teeth obturated with gutta-percha and sealer and exposed to

**Figure 2** No inhibition of growth by 'MGP' gutta-percha.

*Staphylococcus epidermidis* and *Proteus vulgaris* had complete bacterial penetration throughout the entire length of the root canal within 19 days (Torabinejad *et al.* 1990). As root canals may not be completely filled, regardless of obturation technique (Eguchi *et al.* 1985), and sealer may be lost over time (Peters 1986), theoretically, the prognosis of endodontic treatment could be enhanced if the obturation material was able to prevent bacterial recontamination.

'MGP' gutta-percha contains 10% iodoform ($CHI_3$), a crystalline substance, which is soluble in chloroform and ether but has low solubility in water (Budavari 1989). Iodophors, for example, povidone–iodine or 'Betadine', are iodine compounds linked with surface-active agents; these compounds interact with cell walls of microorganisms causing pore formation or generating solid–liquid interfaces at the lipid membrane level, which lead to loss of cytosol material and enzyme denaturation (Fleischer & Reimer 1997, Schreier *et al.* 1997). The product information provided by the manufacturer (Lone Star Technologies, Westport, CT, USA) states that 'the iodoform in the MGP cones is inert until it comes into contact with tissue fluids, which activate the free iodine', and make it available to inhibit bacteria remaining in the canal, or which enter the canal via leakage. Whilst an ideal root-filling material should be bacteriostatic (Grossman 1940), one could speculate that in the clinical situation, the presence of a heavy microbial load, as well as tissue fluids, would dilute the iodine concentration, thereby diminishing the antibacterial effect. Sealer solubility and thickness, shown to influence fluid leakage (Wu *et al.* 1995), would also be expected to moderate access between the medication and microorganisms. Further, given the low solubility of iodoform in water (Budavari 1989), both *in vitro* and *in vivo* quantitative investigations on the release of free iodine from 'MGP' gutta-percha over an extended and clinically relevant time period are indicated.

In the present study, 10% povidone–iodine inhibited all eight bacterial strains examined, whilst the growth of *S. aureus* and *F. nucleatum* only was inhibited by 'MGP' gutta-percha compared to iodoform-free gutta-percha. Similarly, chlorhexidine- and calcium hydroxide-impregnated gutta-percha were less bactericidal than the disinfectants alone (Barthel *et al.* 2002). Whilst the final volume and concentration of free iodine released from the 'MGP' gutta-percha may have differed from that released from paper disks saturated with povidone–iodine, the present results suggest that the release and activation of antimicrobial free iodine from 'MGP' gutta-percha, as claimed by the developers, is not substantial. Based on the present *in vitro* results, reliance on the antimicrobial activity of free iodine released from 'MGP' gutta-percha to disinfect infected root canals cannot be supported.

Iodoform-free gutta-percha cones were used as negative controls; other differences between the iodoform-free gutta-percha and 'MGP' gutta-percha were not investigated but may exist. Interestingly, the growth of two strains, *S. sanguis* and *A. odontolyticus*, was inhibited by iodoform-free gutta-percha. Similarly, traditional gutta-percha cones have been shown to inhibit several bacterial species *in vitro* (Moorer & Genet 1982b, Attin *et al.* 2001), perhaps, in part, because of the inherent antibacterial activity of zinc oxide within the gutta-percha (Moorer & Genet 1982a).

The present data on *S. aureus* correlated with those of the developers. However, none of the studies thus far on 'MGP' gutta-percha using this methodology have adequately quantified zones of inhibition around the gutta-percha. Whilst the clusters of five cones in close proximity (Figs 1 and 2) used in this and the developers' studies may simulate the number of cones used during lateral condensation procedures *in vivo*, the suitability of 'MGP' gutta-percha for 'any obturation technique' (Martin & Martin 1999) has yet to be confirmed. In view of the 120 °C melting point of iodoform (Budavari 1989), and in an effort to permit quantification, further studies could examine the effects, if any, of heat-activated obturation techniques on the antimicrobial characteristics of 'MGP' gutta-percha using gutta-percha disks of standardized diameter.

Finally, Sundqvist *et al.* (1998) reported that the most common isolate from teeth with failing endodontic therapy was *E. faecalis*. In this study, the growth of *E. faecalis* strains was not inhibited by either iodoform-free gutta-percha or 'MGP' gutta-percha in agreement with previous investigators (Silver *et al.* 2000). Duration of presoaking the 'MGP' gutta-percha appeared to be unimportant, as Silver *et al.* (2000) soaked the gutta-percha cones for only 1 min, as opposed to 60 min for this and the developers' investigations. The rationale behind the selection of a 1-h presoaking time to simulate the *in vivo* model is unclear, and the experimental model (Martin & Martin 1999) needs further investigation.

## Conclusion

Iodoform-free gutta-percha inhibited *S. sanguis* and *A. odontolyticus*, whilst 'MGP' gutta-percha inhibited

the growth of *S. aureus*, *S. sanguis*, *A. odontolyticus* and *F. nucleatum*, but not *E. faecalis*, *E. coli* and *P. aeruginosa*. Thus, the addition of iodoform to gutta-percha only had an antimicrobial effect on *S. aureus* and *F. nucleatum* of the eight strains tested. Based on these *in vitro* results, reliance on the antimicrobial activity of free iodine released from 'MGP' gutta-percha to disinfect infected root canals cannot be supported.

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