scientific reports

OPEN

Physicochemical properties of gutta-percha cones before and after a rapid disinfection protocol and its clinical relevance

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This study aimed to evaluate the chemical composition, radiopacity, and antimicrobial activity of five standardized (#25) commercially available gutta-percha points (ProTaper Gold, Zarc, Cerkamed, Autofit, and Reciproc R25), before and after a rapid disinfection protocol. The cones were divided into three groups: untreated (control), treated with sodium hypochlorite, and treated with sodium hypochlorite followed by ethanol rinsing. Significant compositional differences, as assessed by a solvent extraction method, were observed among brands, with gutta-percha content ranging from 11.6 to 17.5%, wax and/or resin from 1.2 to 4%, and inorganic fraction between 74.0 and 83.0%, with ZnO as the predominant component. Radiopacity determined via digital radiography varied among brands and showed a stronger correlation with ZnO content than with $BaSO_4$, challenging the common assumption that barium is the principal radiopacifying agent; Zarc exhibited the highest radiopacity, whereas Cerkamed showed the lowest. None of the gutta-percha points exhibited antimicrobial activity against *Enterococcus faecalis* and *Staphylococcus aureus* under standard clinical conditions, underscoring the necessity of proper disinfection procedures in clinical practice. The disinfection protocols produced minor changes in the wax/resin content of Cerkamed, Reciproc, Autofit, and ProTaper, with the effect being more pronounced in the latter two. In contrast, the metal oxide composition analyzis with wavelength dispersive X-ray fluorescence (WDXRF), showed that only Zark exhibits slight variations in ZnO, $BaSO_4$, SiO_2, and CuO. However, these changes remain insufficient to be considered clinically relevant. Importantly, no effect on the radiographic integrity of the gutta-percha cones was observed, confirming the safety and adequacy of the NaOCl-based disinfection protocols for clinical purposes.

Keywords Gutta-percha, Chemical composition, Radiopacity, Antimicrobial activity, Sodium hypochlorite

The success of endodontic treatments depends on proper clinical practices, which involve several sequential steps: diagnosis of pulp and periapical condition, local anaesthesia, isolation of the rubber dam, access cavity, mechanical and chemical preparation of the root canal system, obturation, and tooth rehabilitation[1]. The biomechanical preparation of the root canal system entails the removal of pulp tissue and debris, elimination of microorganisms, and shaping the canal, all of which are essential for irrigation and subsequent obturation[2]. The latter aims to prevent the accessibility of microorganisms and fluids to the root canal space, including the apical foramen and accessory canals. To ensure a hermetic seal of the root canals, they are filled with an inert sealing material, typically consisting of a combination of a suitable plastic or semi-solid material and an adhesive cement[3]. Ideally, this composite material should have high biocompatibility, sealing capacity, and dimensional stability, as well as good adhesion properties. It should also be radiopaque, insoluble, and immutable in the presence of tissue fluids, bacteriostatic, sterile, and easy to handle and remove (with solvents or heat) in the case of retreatment[3–5]. As gutta-percha (GP) cones meet most of these requirements, they have remained the gold standard filling material for over a century[3,6]. Nonetheless, challenges persist due to poor adhesion to dentin,

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lack of rigidity and length control, and shrinkage during cooling[7,8]. Typical formulations consist of (i) an organic fraction (OF) composed of a GP matrix, a natural polymer (trans-1,4-polyisoprene) with thermoplastic properties extracted from tropical trees of the Sapotaceae family, and minor amounts of waxes and/or resins, which work as plasticizers; (ii) an inorganic fraction (IF) containing the filler zinc oxide (ZnO) with putative antimicrobial properties, and heavy metal sulphates, such as barium sulphate ($BaSO_4$), for radiographic contrast. International standards require gutta-percha cones to be radiopaque, enabling comparison of the density and homogeneity of the root canal filling, and allowing it to be easily distinguished from dental and osseous structures[9]. Existing literature[10,11], attributed an antimicrobial character to the ZnO present in high percentages in the gutta-percha constitution, theoretically due to the release of small particles containing zinc (Zn) from the GP cones, when in contact with water or serum, at neutral pH.

Some manufacturers add a few other chemical agents for antimicrobial effects and coloring purposes[3,6,12]. According to early works[13] with five endodontic filling materials from different manufacturers, the gutta-percha matrix represents about 20% of the total weight, whereas the wax/resins correspond to 1–4%, and the percentages of ZnO and metal sulphates can vary between 59 and 75% and 3–17%, respectively. Subsequent studies have shown that the chemical composition of some other brands of gutta-percha falls well outside these ranges, but the overall trend remains roughly the same[3,14,15]. Not surprisingly, the nature and proportion of each component affect the clinical and mechanical properties of (dental) GP cones[6,16]. Zinc oxide (ZnO), for example, influences the brittleness, elongation, and tensile strength of the filling material[6]. However, most GP brands do not specify the chemical composition of the cones, which hinders clinicians from predicting interactions (and possible outcomes) through the simple analysis of a material data sheet. The available information comes mainly from a surprisingly small number of research works, given the long history of GP materials in dentistry.

In the endodontic practice, different irrigants are used, such as natural agents (green tea or Triphala) and chemical agents, such as sodium hypochlorite (NaOCl), which has antimicrobial capacity and organic tissue dissolution, and chlorhexidine digluconate (CHX), which presents antimicrobial effectiveness against Gram-positive and Gram-negative bacteria[17–19]. Also, within chemical substances, chelating agents, such as ethylenediaminetetraacetic acid (EDTA) and citric acid (CA), can remove the inorganic part of the smear layer defined as a superficial layer of debris retained on dentin and other surfaces after mechanical instrumentation as irrigants[18]. Sodium hypochlorite (NaOCl) is considered close to an ideal irrigant due to its broad-spectrum germicidal and fungicidal properties, its effectiveness even in the presence of blood, plasma, and tissue-derived proteins, as well as its strong organic tissue-dissolving capability. It exhibits low surface tension, which facilitates penetration into dentinal tubules and does not cause tooth discoloration. Additionally, NaOCl is cost-effective, easy to handle, and readily available in clinical settings[20,21]. Despite these advantages, NaOCl alone does not completely fulfil all characteristics of an ideal irrigant, such as complete removal of the smear layer, absolute biocompatibility, and absence of potential cytotoxic effects on periapical tissues. Therefore, it is commonly used combined with other irrigating solutions to achieve optimal clinical outcomes[20]. Reactions like saponification, amino acid neutralization, and chloramination, that occur in the presence of organic tissues and microorganisms, promote antimicrobial action and tissue dissolution[22]. The effectiveness of its action is related to the exposure time, volume, concentration of the solution, temperature, and agitation[17,23]. Typically, NaOCl is used at concentrations between 0.5 and 6%. Although higher concentrations lead to a higher tissue dissolution rate, lower concentrations can be compensated for by increasing the volume applied, thus achieving the same dissolution power of organic tissue[18,22].

Given the antimicrobial characteristics of NaOCl and its widespread use as an irrigant, it is also used to disinfect GP cones before their placement in the root canal[24]. Although gutta-percha cones are manufactured under aseptic conditions, they are easily contaminated due to inadequate storage, physical handling, and contact with aerosols[25]. Unfortunately, the thermoplastic character of the GP cones makes this material impossible to sterilize by conventional autoclave processes, which would cause the material's deformation. Due to this limitation, the use of NaOCl as a rapid decontamination agent was proposed, demonstrating that after bacterial contamination of the cones by *Staphylococcus epidermidis*, *Corynebacterium xerosis*, *Escherichia coli*, and *Enterococcus faecalis*, and their subsequent immersion in Clorox (5.25% NaOCl) for 30 to 60 s, no subsequent bacterial growth was observed at the macroscopic level[24]. In another study, it was concluded that the immersion of GP cones for 1 min. in 5.25% NaOCl, enables disinfection against Gram-positive and Gram-negative microorganisms and spores[26]. It should be noted that the contact of NaOCl with the surface of gutta-percha cones promotes the formation of hypochlorite crystals that should be removed by washing with alcohol or distilled water because these crystals interfere with the sealing of the root canals[27].

As ZnO is the main component of GP cones to which an antimicrobial capacity was attributed, the clinical community has been questioning the prior need for GP decontamination in clinical practice[10,11].

Therefore, this study aimed to evaluate the potential influence of a NaOCl-based disinfection protocol, commonly used in clinical practice, on the chemical composition, antimicrobial activity, and radiopacity of five different brands of GP cones. Additionally, the original chemical composition of these commercial GP cones was compared among brands and correlated with their radiopacity.

Materials and methods

Reagents and solutions

For the GP chemical analysis, the following reagents were used: chloroform (99.8%) from the Fisher Chemical brand, acetone (99.5%) purchased from Chem-Lab NV, 96% ethanol and 5.25% NaOCl, and deionized water. For the antimicrobial assays, the bacteria *Enterococcus faecalis* CECT 481 and *Staphylococcus aureus* ATCC 6538 were employed, using Muller Hinton and Tryptic Soy Agar (TSA) culture media.

Gutta-percha cones and other materials

Endodontic gutta-percha cones from five commercially available brands in Portugal were evaluated. The brands included Autofit 6%, Cerkamed 6%, Reciproc R25 (VDW), ProTaper Gold F2 (Dentsply Sirona), and Zarc Z4 BlueShaper 6%, all in size n. 25. Detailed information regarding each brand, including lot numbers and expiration dates, is presented in Table S1 of the Supplementary Material. The gutta-percha cones were divided into 3 groups:

- Group C: untreated gutta-percha cones, i.e., without any type of disinfection treatment (control).
- Group S: Gutta-percha cones disinfected with 10 mL of 5.25% sodium hypochlorite, in a Petri dish, for 1 min.
- Group A: Gutta-percha cones disinfected with 10 mL of 5.25% sodium hypochlorite in a Petri dish for 1 min. (as in S group) and then rinsed with 96% alcohol (ethanol) for 1 min.

Each gutta-percha cone was treated individually, in separate Petri dishes, to prevent any contamination. After washing, the cones were air-dried on sterile gauze.

Chemical analysis

The composition of the organic and inorganic fractions of the GP cones was determined using the protocols described by Friedman[13]. Briefly, 1.0 g of commercial GP points were placed in previously tared chloroform-compatible centrifuge tubes and dissolved in 10 mL of chloroform for 24 h, under magnetic stirring, using a stir plate from LBX Instruments, H03D series; the resulting mixture was centrifuged for 15 min. at 12,000 *g*, with a centrifuge Eppendorf 5810 (rotor F-24-6-28). This allowed for the separation of the solid phase (inorganic components: zinc oxide and metal sulphates) from the supernatant (organic fraction: GP, resins, and waxes). After decantation, the GP component was coagulated by adding 10 mL of acetone and separated by filtration using a vacuum pump (KNF N022AT.18) and filter paper (filters RS), and then dried inside a desiccator for 12 h. After acetone evaporation, the GP fraction was weighed with an analytical balance (Ohaus Pioneer). The remaining chloroform-soluble components (wax/resin) were recovered by heating the filtrate in a beaker at 60 °C, using a heater (LBX Instruments H03D), until complete solvent evaporation; the mass of the resulting solid residue was assessed by differential weighing. All assays were repeated six times ($n = 6$), and results are presented as the mean of the six independent replicates.

WDXRF analysis

The elemental composition was determined using wavelength dispersive X-ray fluorescence (WDXRF), a non-destructive technique that allows direct multi-element analysis of solid materials. To obtain a gutta-percha agglomerate, 1.0 g of GP cones were thermoplasticized using the gutta-percha obturation system - SuperEndo-beta - at a temperature of 200 °C. This agglomerate was then subjected to a force of 10 tons using a hydraulic press, Specac, PT. No. 3165, producing a uniform circular “pellet” with a diameter of about 2 cm and a thickness of 5 mm. The “pellet” was placed in steel sample holders with an opening diameter of 8 mm, and the elemental composition of each sample was performed directly, under vacuum with a 4 kW power, using the S4 Pioneer Bruker AXS equipment. The evaluation model considered the different elements bound to oxygen in the form of oxides, whose concentrations were determined using quantitative universal calibrations (“standardless measurement”), which was appropriate given the expected high concentrations and counting errors of less than 5%. Each assay was conducted with five independent replicates ($n = 5$), and data are reported as the mean value from these replicates.

Radiopacity analysis

For the radiopacity analysis, 15 polymethylmethacrylate (PMMA) acrylic blocks were instrumented using the Zarc BlueShaper System (Z1-Z4), finishing with an apical gauge at D0 of 0.25 mm. The GP cones calibration was performed with a calibrated ruler (Woodpecker R1 Plus); all brands were calibrated to 0.25 mm.

To maintain the same experimental conditions, a support was made to hold the Aluminium stepwedge (Al), the acrylic block, and the phosphor film (PSPIX), so that they would remain in the same position. The X-ray focus detector was positioned 27 cm from the support.

Digital images were evaluated at the junction of the apical and middle thirds using GIMP 2.0. Grayscale values (0 [black] to 255 [white]) were quantified in specific regions of interest through the histogram tool, allowing for the assessment of radiographic density. Radiopacity was then determined by comparing these grayscale measurements with those obtained from the Aluminium stepwedge. All assays were performed in triplicate ($n = 3$), and results are presented as the mean of the three independent replicates.

Antimicrobial analysis

Suspensions of each microorganism were prepared in sterile distilled water at a concentration of 1×10^8 cfu/mL (turbidity of 1.0 on the McFarland scale), under aseptic conditions using a laminar flow cabinet (Ninolabinterior, ninoSAFE class II 1200 Value). These *E. faecalis* and *S. aureus* suspensions were inoculated in Müller-Hinton agar plaques by spread plating. The plaques were incubated at 37 °C for 24 h. Then, 3 gutta-percha cones from the same brand, one for each study group, were placed inside individual inoculated agar plaques. The plaques were incubated again at 37 °C for 24 h. All assays were repeated in triplicate ($n = 3$).

The presence or absence of halos around each cone placed in the solid medium plaques was observed to assess growth inhibition.

Data analysis

Data were analysed with the software package IBM SPSS Statistics (Armonk, NY, USA), by applying both descriptive and inferential statistical procedures. Inferential comparative analyses of mean values were performed using One-way ANOVA, followed, when applicable, by Tukey's post-hoc test. The ANOVA's assumptions were verified and validated. A 5% significance level was adopted for all inferential analyses.

Results and discussion

Chemical composition of gutta-percha cones

In this study, we analyzed the chemical composition of five commercial brands (ProTaper Gold, Zarc, Cerkamed, Autofit, and Reciproc R25) of standardized gutta-percha cones n. 25, in the as-purchased form (Group C), after undergoing a rapid disinfection protocol (immersion in 5.25% NaOCl; Group S), and following a second cleaning step of rinsing with alcohol (Group A). First, the organic (gutta-percha and waxes/resins) and inorganic fractions were separated and analyzed quantitatively using the extraction method described in the Experimental Section. As shown in Table 1, the recovered mass values are quite high, ranging between 96.1% and 98.2%, which demonstrates the extraction efficacy. Overall, significant differences were observed among the five gutta-percha brands regarding their chemical composition. The inorganic fraction ranged between 74.0% (ProTaper) and 83.0% (Autofit), highlighting Autofit as having the highest inorganic content, which might confer greater rigidity, beneficial specifically in lateral condensation techniques, consistent with previous findings reported in the literature[28].

The total organic fraction varied from 14.8% (Autofit) up to 22.5% (ProTaper), indicating substantial variability among manufacturers. Regarding the pure GP content, Autofit and ProTaper exhibited the highest percentages (17.2% and 17.5%, respectively), suggesting superior plasticity and adaptability for clinical applications, as indicated by prior research works[14,16,29], emphasizing the importance of chemical composition variations on clinical outcomes[15,16,30,31], and underlining the importance of clinicians selecting GP points based on the intended endodontic technique, as variations in composition may directly influence clinical performance.

Except for Zark, slight variations were detected in the wax and resin content, following the rapid disinfection of cones with 5.25% NaOCl (Group S) and subsequent alcohol rinsing (Group A). Although some of these differences reached statistical significance (Table 2), they are small and generally fall within the margin of error. Only the relative variations observed for Autofit and Protaper are more pronounced; however, they remain

Fraction	Brand	Mean (%) (SD)	*p*
RMV	Zark	97 ± 1	0.129
	Reciproc	98 ± 2	
	Cerkamed	97.7 ± 0.2	
	Autofit	98 ± 2	
	Protaper	96 ± 2	
IF	Zark	80.2 ± 0.2^{b}	< 0.001
	Reciproc	79.5 ± 0.1^{b}	
	Cerkamed	79.0 ± 0.8^{b}	
	Autofit	83.0 ± 0.5^{c}	
	Protaper	74.0 ± 0.7^{a}	
GP	Zark	14 ± 2^{b}	< 0.001
	Reciproc	$16 \pm 2^{b,c}$	
	Cerkamed	17 ± 1^{c}	
	Autofit	11.6 ± 0.6^{a}	
	Protaper	17.5 ± 0.8^{c}	
W & R	Zark	1.2 ± 0.3^{a}	< 0.001
	Reciproc	$2.0 \pm 0.1^{a,b}$	
	Cerkamed	1.5 ± 0.4^{a}	
	Autofit	$3 \pm 1^{b,c}$	
	Protaper	4 ± 1^{c}	
OF	Zark	$16 \pm 2^{a,b}$	< 0.001
	Reciproc	$18 \pm 2^{a,b}$	
	Cerkamed	18.7 ± 0.9^{b}	
	Autofit	15 ± 2^{a}	
	Protaper	22 ± 2.0^{c}	

Table 1. Chemical composition (%±SD) of gutta-percha points ($n = 6$) from five commercial brands in Group C. Results are presented as mean percentages (%) and corresponding standard deviations (SD). RVM = Recovered Mass Values; IF = Inorganic Fraction; GP = Gutta-Percha; W&R = Waxes and Resins; OF = Organic Fraction. Different superscript lowercase letters indicate statistically significant differences ($p < 0.05$, ANOVA followed by Tukey's post hoc test).

Brands	Treatment	Mean (%) (IF)	*p*	Mean (%) (GP)	*p*	Mean (%) (W&*R*)	*p*	Mean (%) (OF)	*p*
Zark	C	80.2 ± 0.2	0.081	14 ± 2	0.599	1.2 ± 0.3	0.551	16 ± 2	0.757
	S	80.7 ± 0.4		14 ± 2		1.3 ± 0.4		16 ± 2	
	A	81.0 ± 0.6		15 ± 1		2 ± 2		16 ± 2	
Reciproc	C	79.5 ± 0.1	0.311	16 ± 2	0.478	2.0 ± 0.1 ^{a}	0.005	18 ± 2	0.488
	S	79.8 ± 0.5		16 ± 2		2.7 ± 0.3 ^{b}		18 ± 2	
	A	82 ± 4		17 ± 2		2.4 ± 0.4 ^{a,b}		19 ± 3	
Cerkamed	C	79.0 ± 0.8	0.266	17 ± 1	0.493	1.5 ± 0.4 ^{a,b}	0.042	18.7 ± 0.9	0.727
	S	79.4 ± 0.4		17 ± 2		1.9 ± 0.5 ^{b}		20 ± 3	
	A	79.5 ± 0.3		18 ± 1		1.3 ± 0.2 ^{a}		19 ± 1	
Autofit	C	83.0 ± 0.5	0.879	11.6 ± 0.6	0.281	3 ± 1 ^{a}	0.030	15 ± 2	0.644
	S	83.0 ± 0.4		12 ± 2		1.7 ± 0.1 ^{b}		14 ± 2	
	A	83.1 ± 0.2		13 ± 2		1.8 ± 0.3 ^{a,b}		15 ± 2	
Protaper	C	74.0 ± 0.7	0.437	17.5 ± 0.8	0.187	4 ± 1 ^{a}	0.003	22 ± 2	0.165
	S	75 ± 2		20 ± 3		3.5 ± 0.9 ^{a}		24 ± 3	
	A	75 ± 1		19 ± 2		2 ± 1 ^{b}		21 ± 3	

Table 2. Comparative chemical composition (%±SD) of gutta-percha points ($n = 6$) from five commercial brands across the three study groups (C = control, S = sodium hypochlorite, A = sodium hypochlorite + alcohol). Results are shown as mean values ± standard deviation (SD). Different superscript lowercase letters indicate statistically significant differences ($p < 0.05$, ANOVA followed by Tukey's post hoc test). IF: inorganic fraction; GP: gutta-percha: W & R: waxes & resins; OF: organic fraction.

Brand	ZnO (%)	$BaSO_4$ (%)	Na_2O (%)	TiO_2 (%)	MgO (%)	SiO_2 (%)	CuO (%)
Zarc	60 ± 1 ^{a}	16.5 ± 0.2 ^{ab}	13.6 ± 0.7 ^{a}	3.9 ± 0.1 ^{ab}	1.7 ± 0.3 ^{a}	1.8 ± 0.5 ^{a}	0.074 ± 0.005
Protaper	59 ± 1 ^{a}	19 ± 4 ^{a}	10 ± 2 ^{b}	3.6 ± 0.4 ^{a}	1.2 ± 0.2 ^{ab}	2.0 ± 0.4 ^{a}	0.08 ± 0.02
Reciproc	76 ± 3 ^{b}	6 ± 2 ^{d}	15 ± 1 ^{a}	0.2 ± 0.2 ^{c}	0.6 ± 0.7 ^{ab}	0.9 ± 0.6 ^{ab}	0.075 ± 0.003
Autofit	68 ± 2 ^{c}	11 ± 2 ^{cd}	15.3 ± 0.8 ^{a}	ND	0.2 ± 0.4 ^{b}	ND	0.078 ± 0.002
Cerkamed	59 ± 4 ^{a}	13 ± 4 ^{bc}	11 ± 1 ^{b}	6 ± 2 ^{b}	4 ± 1 ^{c}	3.6 ± 0.8 ^{c}	0.083 ± 0.003
p	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.472

Table 3. Elemental composition (%±SD) of gutta-percha points (Group C) as determined with WDXRF ($n = 5$). Different superscript letters indicate statistically significant differences between brands ($p < 0.05$, ANOVA followed by Tukey's post hoc test). ND = not detected.

insufficient to be considered clinically relevant. Worth noting, a mass increase was observed in a few cases from the C to the S/A groups. Such variations may be artefactual, as the solvent was evaporated at 60 °C—a temperature close to the typical melting point of waxes and approaching their boiling point (> 75 °C). Although the risk of volatilization is likely minimal, the increase in vapor pressure at this temperature may have resulted in slight wax evaporation and random partial mass losses.

The inorganic and organic fractions remained unchanged in all brands, confirming the overall chemical stability of GP cones after disinfection. Taken together, these findings reinforce previous reports suggesting that short-term exposure to NaOCl does not significantly alter the initial chemical composition of GP cones[32].

WDXRF analysis

A standardless WDXRF analysis was conducted on GP cones under the three conditions studied (Groups C, S, and A). Table 3 shows the elemental composition of the five commercial brands, pointing out ZnO, $BaSO_4$, Na_2O, TiO_2, MgO, SiO_2, and CuO as the predominant oxides. ZnO is the primary filler in all GP brands, ranging from 58.9% to 76.2%, in agreement with results reported in other works[33,34]. Reciproc excels with the highest concentration amongst all brands under study (76.2%), probably enhancing the cones' rigidity. The second most abundant metal is Barium (as $BaSO_4$), whose concentration ranged from 5.9% to 19.2%, in accordance with previous studies. [13] Interestingly, Reciproc stands out with the lowest percentage; however, this does not correlate with its ability to generate radiographic contrast, as discussed in the next section.

The comparison of the elemental composition between the three studied groups (C, S, and A), revealed that the rapid disinfection protocol did not result in statistically significant differences for most brands (Supplementary Material, Tables S2 to S5). Therefore, the observed variations are considered negligible from a clinical perspective. As shown in Table 4, only Zarc exhibited statistically significant changes in ZnO, $BaSO_4$, SiO_2, and CuO contents.

Treatment	ZnO (%)	$BaSO_4$ (%)	Na_2O (%)	TiO_2 (%)	MgO (%)	SiO_2 (%)	CuO (%)
C	60 ± 1 [a]	16.5 ± 0.2 [a]	13.6 ± 0.7	3.9 ± 0.1	1.7 ± 0.3	1.8 ± 0.5 [a]	0.074 ± 0.005
S	61.2 ± 0.5 [ab]	16.5 ± 0.2 [a]	13.5 ± 0.5	3.9 ± 0.1	1.7 ± 0.4	1.5 ± 0.5 [ab]	0.076 ± 0.002
A	62.4 ± 0.4 [b]	16.9 ± 0.1 [b]	13.3 ± 0.4	4.00 ± 0.04	1.3 ± 0.1	0.9 ± 0.1 [b]	0.081 ± 0.002
p	0.021	0.006	0.570	0.165	0.127	0.020	0.034

Table 4. Elemental composition (%±SD) of Zarc gutta-percha points ($n = 5$) across three treatment groups (C, S, A), with relative values of ZnO, $BaSO_4$, Na_2O, TiO_2, MgO, SiO_2, and CuO, as obtained with WDXRF. Statistically significant differences are indicated by different superscript letters ($p < 0.05$, ANOVA followed by Tukey's post hoc test).

Brand	Mean Radiopacity (Unit ± SD)
Cerkamed	125.1 ± 0.5
ProTaper Gold	129.1 ± 0.5
Autofit	130.2 ± 0.5
Reciproc	132.6 ± 0.5
Zarc	135.5 ± 0.5

Table 5. Mean radiopacity values (± SD) of gutta-percha points ($n = 3$) from five commercial brands in Group C.

Group	Groups	*p*
Control Group (C)	Sodium Hypochlorite (S)	0.650
	Sodium Hypochlorite + Alcohol (A)	0.329
Sodium Hypochlorite (S)	Control Group (C)	0.650
	Sodium Hypochlorite + Alcohol (A)	0.650
Sodium hypochlorite + Alcohol (A)	Control Group (C)	0.329
	Sodium Hypochlorite (S)	0.601

Table 6. Statistical (pairwise) comparison of radiopacity values between experimental groups (C = control, S = NaOCl, A = NaOCl + alcohol); $n = 3$.

Unexpectedly, a trend toward increased percentages of some metal oxides from group C to groups S and A was observed, possibly due to the slight dissolution of waxes by the extraction solvents. Nevertheless, such alterations are generally small in absolute terms and are only relatively pronounced in the case of SiO_2 (less 50%), which is a minor component of the cones' material. Overall, we consider these changes to be not clinically relevant.

These outcomes differ slightly from previous studies, which indicated that alterations in the metal oxide content of GP point could occur depending on factors such as sodium hypochlorite concentration and the duration of immersion[35]. However, it is important to note that the experimental conditions evaluated in such studies differed significantly from those routinely employed in clinical practice, particularly regarding concentrations and exposure times, thus limiting the direct applicability and clinical relevance of those findings.

Radiopacity

Radiopacity is critical for the accurate assessment of obturation quality in clinical practice. As shown in Table 5, Zarc has the highest radiopacity, whereas Cerkamed has the lowest. A statistical analysis comparing radiopacity data across the five brands shows that, except for the Autofit/ProTaper pair, the observed differences are statistically significant (Supplementary Material, Table S6).

Interestingly, the relative levels of radiopacity within the five cone brands do not correlate with the corresponding contents in $BaSO_4$, which is commonly assumed to be the primary radiopacifier. For example, while ProTaper has the highest content of this heavy metal, it shows the second lowest radiopacity among the five GP brands. Conversely, Reciproc presents the lowest percentage of $BaSO_4$, but it ranks in second place in terms of radiopacity. On the other hand, it shows the highest amount of ZnO among all the studied brands. The strong correlation between ZnO percentages and radiopacity values shown in the GP brands studied in our work suggests that this metal oxide significantly contributes to radiopacity.

Regarding the effect of disinfection and subsequent cleaning treatments on radiopacity, no significant differences were observed across the brands tested (Table 6). This clinically relevant finding ensures that the diagnostic reliability of GP cones remains unchanged after routine rapid disinfection, maintaining their utility and diagnostic accuracy in the post-treatment phase.

Antimicrobial analysis

This study evaluated the antimicrobial efficacy of five gutta-percha brands (Cerkamed, ProTaper Gold, Reciproc R25, Autofit, and Zarc), both before and after applying the disinfection protocol. Results revealed that, following incubation for 24 h at 37 °C in Mueller-Hinton media, none of the tested gutta-percha cones exhibited antimicrobial activity against *E. faecalis* or *S. aureus* (as an example, Figure S1 shows the results obtained with Cerkamed). These findings contrast with earlier reports suggesting that gutta-percha points exhibit antimicrobial properties due to zinc oxide release. [10,11] However, methodological differences must be considered when comparing these results with earlier research works. Previous studies utilized modified laboratory conditions, including diluted nutrients, lower incubation temperatures, and serum as the sole nutrient source, aiming to mimic oral cavity conditions. Such modifications, however, diverge from current Clinical and Laboratory Standards Institute (CLSI) guidelines, limiting their clinical applicability and comparability. Additionally, prior studies indicated increased zinc oxide release upon prolonged contact of gutta-percha cones with liquid environments. [10,11] Clinically, this scenario is unlikely, as GP is typically combined with resin-based endodontic sealers, preventing direct contact with aqueous environments and thereby inhibiting the release of zinc oxide.

Supporting our findings, other works demonstrated that conventional GP cones lack antimicrobial properties, whereas GP impregnated with chlorhexidine or calcium hydroxide-based pastes exhibited significant antimicrobial effects[36]. This reinforces the conclusion that conventional GP cones do not provide clinically relevant antimicrobial action per se.

Clinically, these results underscore the critical importance of implementing a rapid disinfection protocol, such as sodium hypochlorite treatment, before the clinical use of GP points, given the thermoplastic nature of GP, which precludes heat sterilization methods. [24]

Conclusions

Gutta-percha cones are considered the standard endodontic material for root canal obturation.This study enabled a detailed characterization of five commercial brands — Cerkamed, ProTaper Gold, Reciproc R25, Autofit, and Zarc — evaluating their chemical composition, elemental content, radiopacity, and antimicrobial activity, both before and after undergoing a rapid chemical disinfection protocol. Significant differences were observed in the chemical composition and radiopacity between brands in their as-purchased form (control group C). Variations in the organic and inorganic fractions were detected, with changes in wax and resin content identified across all brands except Zarc. Additionally, elemental analysis showed differences in the relative abundances of ZnO, $BaSO_4$, and SiO_2, especially in Zarc. Interestingly, radiopacity was found to correlate more strongly with ZnO content than with $BaSO_4$, challenging the traditional assumption that $BaSO_4$ is the primary radiopacifier. These findings corroborate with previous literature, suggesting a more substantial role of ZnO in radiopacity.

None of the evaluated GP brands showed antimicrobial activity against *E. faecalis* or *S. aureus*. This important observation contradicts the long-standing belief that ZnO contributes to the intrinsic antimicrobial properties of GP points and reinforces the need for proper aseptic handling and disinfection before clinical use. Following disinfection with NaOCl and ethanol, minor but statistically significant changes were observed in the wax and resin content of all brands, except for Zark, which also exhibited small variations in metal oxide content. Nevertheless, these alterations should not compromise their essential properties, such as radiopacity. These findings uphold the need, and at the same time, clinical safety and effectiveness, of NaOCl-based disinfection protocols in preserving the functional integrity of GP cones.

For future studies, it is crucial to explore the correlation between chemical composition and the physical and thermal behaviour of gutta-percha, as well as to better understand the role of minor components such as waxes and resins. The compositional variations observed, particularly in the Zarc brand, warrant deeper investigation into their potential clinical implications.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Received: 17 January 2026; Accepted: 8 April 2026
Published online: 12 April 2026

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Acknowledgements

This work was financed by national funds through the FCT - Foundation for Science and Technology, I.P., under the scope of the projects UIDB/04585/2025 of the Research Center - CiiEM), UID/04378/2025 and UID/PRR/04378/2025 of the Research Unit on Applied Molecular Biosciences - UCIBIO, and LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB.

Author contributions

M. M.: Writing – original draft, Methodology, Investigation, Formal analysis. J. B.: Methodology. L.P.: Formal analysis. H.B.: Methodology. J.A.N.: Methodology, Conceptualization. M.G.A.: Supervision, Methodology, Conceptualization. All authors reviewed the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-026-48573-5.

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