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An international survey on the use of calcium silicate-based sealers in non surgical endodontic treatment

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Abstract

Objectives: To gain insight on the current clinical usage of Bioceramic root canal sealers (BRCS) by general dental practitioners (GDPs) and endodontic practitioners (EPs) and to determine if BRCS clinical application is in accordance with the best available evidence.

Material and methods: An online questionnaire of 18 questions addressing BRCS was proposed to 2335 dentists via a web-based educational forum. Participants were asked about socio-demographic data, clinical practice with BRCS and their motivation for using BRCS. Statistical analysis (Chi-squared test or Fisher's exact test) were applied, as appropriate, to assess the association between the variable categories (p -value <0.05).

Results: The response rate was 28.91%. Among respondents, 94.8% knew BRCS (EPs more than GDPs, $p<0.05$) and 51.70% were using BRCS. The primary reason for using BRCS was their believe of its improved properties (87.7%). Among BRCS users, single cone technique (SCT) was the most employed obturation method (63.3%) which was more applied by GDPs ($p<0.05$); EPs utilized more of the thermoplasticized obturation techniques ($p<0.05$). A proportion of 38.4% of BRCS users indicated the usage of SCT with BRCS regardless of the root canal anatomy (GDPs more than EPs $p<0.05$) and 55.6% considered that BRCS may influence their ability to re-establish apical patency during retreatment (GDPs more than EPs $p<0.05$).

Conclusions: This study highlights wide variation in the clinical use of BRCS which is not in accordance with the current literature.

Clinical relevance: This inconsistency among EPs and GDPs on BRCS clinical application requires further clarifications to better standardize their use and improve their future evaluation.

Keywords: Bioceramic root canal sealer, calcium silicate-based sealer, endodontics, root canal filling, single cone technique.

Introduction

1 The primary goal of endodontic treatment is to prevent and to treat apical periodontitis by adequately
2 disinfecting and filling the root canal space [1]. Since reaching a sterile environment is impossible due
3 to the complexity of the root canal system, filling and sealing of the latter is essential to prevent the
4 passage of residual microorganisms and their by-products into the peri-radicular tissues [2]. Several
5 obturation techniques combining gutta-percha and a root canal sealer have been used to seal the root
6 canal space including the “single cone technique” (SCT) [3]. This technique relies primarily on one
7 gutta-percha cone and more emphasis is placed on the sealer which functions as a root canal filler.
8 Among the available root filling techniques, SCT is considered to be less technique-sensitive as well
9 as cost-effective [4]. However, a higher sealer volume inside the root canal space may negatively
10 influence the seal as most available sealers tend to shrink upon setting [5]. As a result, SCT combined
11 with conventional sealers was deemed inappropriate, and up till now it was recommended to maximize
12 the gutta percha volume and minimize the sealer thickness [6] using thermoplasticized gutta-percha
13 obturation techniques. Since Mineral Trioxide Aggregate development, silicate-based materials are
14 widely used in endodontic procedures because of their excellent biological properties [7]. This explains
15 the advent of calcium silicate-based sealers also known as bioceramic root canal sealers (BRCS) [8]
16 characterized by handling and viscosity properties optimized for obturation of the root canal space.
17 Recently, SCT has been updated with BRCS usage considering that the latter do not shrink upon
18 setting [9]. On the other hand, thermoplasticized gutta-percha obturation techniques are controversial
19 with this type of sealers [10,11].

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30 Given the increasing BRCS release into the market, an international survey was set up to examine the
31 knowledge and clinical application of these materials by endodontic practitioners (EPs) and general
32 dental practitioners (GDPs) in order to determine if the latter were in accordance with the best
33 available evidence.
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Material and methods

1 A descriptive, cross-sectional and international survey was conducted online in November 2017 with
2 two recalls set 15 days apart. A multiple-choice self-administered questionnaire was sent by e-mail to
3 all members identified as dentists on a web-based educational forum, "Endolit". At the time of the
4 investigation, it represented 2335 practitioners of whom 62.4% were registered on this forum as
5 endodontic specialists, 11.1% as endodontic residents and 26.5% as GDPs. This close-ended
6 questionnaire included 18 questions related to BRCS endodontic use (Fig. 1). All participants were
7 asked to provide socio-demographic data on their age, gender, practice experience, type of practice
8 and geographic region. Other questions focused on dentists' knowledge about BRCS, the way they
9 apply the material during endodontic treatment, as well as their motivation and perception toward
10 BRCS. All participants received a written explanation of the terms of the study prior to their
11 participation and had to give their informed consent to participate in the study without any financial
12 compensation. This survey was approved by the Ethics Committee of Aix-Marseille University, France
13 (reference 2018-06-02-002).
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Statistical analysis

22 Statistical analysis was performed using SPSS Statistics (IBM, Armonk, USA). The participants of the
23 present study were categorized into two groups; General dental practitioners (GDPs) or Endodontic
24 practitioners (EPs). The EPs group included both endodontic specialists and endodontic residents
25 considering that their undergoing specialty training, education and clinical practice are monitored by
26 endodontic specialists. First, the distribution by age (5 classes), gender, years of practice (4 classes)
27 and type of practice (GDPs/EPs) was examined with regard to the following two points: "has heard of
28 BRCS before" and "has already used BRCS". The rest of the analysis was conducted only with the
29 respondents who reported using BRCS at a frequency ranging from "sometimes" to "always" (Fig. 1).
30 In this part, we were particularly interested in the influence of the type of practice (GDPs VS EPs) on
31 clinical habits with BRCS usage, and the perception about the material. Chi-squared test (χ^2) or
32 Fisher's exact test were used, as appropriate, to assess the associations between the variable
33 categories (p -value<0.05).
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Results

Characteristics of dentists included in the study

Of the 2335 contacted dentists, 675 practitioners gave their informed consent yielding a response rate of 28.9%. Participants were from: North America (50.8%), Europe (22.8%), Asia/Australia (16.3%), South America (5.9%) and Africa (4.2%). The age of the participants ranged from 23 to 85 years [mean±standard deviation (SD): 43.9±13; median=41] and years of practice ranged from 0 to 62 years [mean±SD: 18.1±12.9; median=15]; 80.3% of them were EPs and 19.7% GDPs (Table 1).

Knowledge and usage of BRCS

Among the participants (n=675), 94.8% had heard about BRCS before (n=640), which was significantly associated with being in the EPs group (p<0.05) (Table 2). Participants knew about BRCS via: “conferences/course” (57.50%), followed by “manufacturers’ speech/advertising” (22.2%), “colleagues’ experience” (12.2%), “literature” (4.1%) and “websites/internet” (1.5%).

Among the respondents knowing BRCS, 58.4% had used BRCS at least once (n=374), which was significantly associated with being in the EPs group (p<0.05) (Table 2). These latter were further asked how often they used BRCS in their daily practice (Fig. 1). The distribution of the frequency of BRCS usage was: “always” (22.2%), “frequently” (22.4%), “sometimes” (48.7%) and “never” (6.7%) and couldn’t be related to the type of practice (p>0.05) (Table 2). For the subsequent statistical analysis, those describing a BRCS usage ranging from “sometimes” to “always” were identified as “BRCS users” (n=349) and had to answer questions related to their clinical practice with BRCS and reasons for using them. This subgroup represented 51.7% of the overall participants and was composed of 87.4% EPs and 12.6% GDPs.

BRCS clinical practice

Canal drying

Among BRCS users, 69.4% employed paper points alone to dry canals prior to obturation, 19.6% used vacuum and paper points, 10.1% used alcohol before paper points and 0.9% used vacuum alone.

BRCS application

For BRCS application, 51% of BRCS users employed a device helping injection and distribution of the sealer into the root canal (either a Lentulo spiral 8%, a specific injection tip 40.7%, a file 2% or a sonic/ultrasonic system 0.3%). The remaining 49% carried out a simple coating of the master cone with BRCS.

Obturation technique with BRCS

Among BRCS users, 63.3% employed SCT for root canal obturation; 16.7% used cold lateral compaction and 46.1% used thermoplasticized gutta-percha obturation technique (either carrier-based obturation or warm vertical compaction). **Systematic use of SCT** with BRCS was reported by 40.7% of BRCS users. There was a significant association between the obturation method and the type of practice: GDPs tended to use SCT more (p<0.05) while EPs used warm obturation techniques more often (p<0.05) (Fig. 2). **BRCS users** were also asked about the obturation methods they used with

1 other sealers than BRCS. Our results highlighted that the preferential obturation method significantly
2 changed depending on the type of sealer used, independently of the type of practice. SCT was the
3 most implemented technique with BRCS ($p<0.05$) while warm vertical compaction was preferentially
4 used with other types of sealers ($p<0.05$) (Fig. 3). Specific bioceramic nanoparticles pre-
5 impregnated/pre-coated gutta-percha cones were used by only 22.1% of the BRCS users. There was
6 no significant association between using these specific cones and the type of practice ($p>0.05$).
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10 **Motivations and perception toward BRCS**

11 The improved biological and physicochemical properties appeared as the main reason for using
12 BRCS, followed by the possibility to fill simultaneously the root canal space and a perforation. These
13 two motivations were not associated with the type of practice. On the other hand, reasons such as the
14 innovative aspect of BRCS, the simplification of clinical procedures, the connection with minimal
15 invasive dentistry (preservation of coronal, pericervical and radicular dental tissue) and the fact that no
16 specific equipment for obturation would be needed all showed significant association with being a
17 GDP ($p<0.05$) (Table 3).
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22 To the question *“Do you think BRCS usage with SCT can be indicated regardless of the root canal*
23 *anatomy?”*, 38.4% of the BRCS users answered “Yes”, 57.6% answered “No” and 4% had no opinion.
24 Thinking that BRCS usage with SCT could be indicated regardless of the root canal anatomy was
25 significantly associated with being in the GDPs group (68.2%) rather than in the EPs group (34.1%)
26 ($p<0.05$).
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30 To the question *“Do you think BRCS usage may influence the ability to re-establish apical patency*
31 *during non-surgical retreatment?”*, 55.6% of the BRCS users answered “Yes” and it was significantly
32 associated with being in the GDPs group (68.2%) rather than in the EPs group (51.8%) ($p<0.05$).
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Discussion

1 The present study aimed to gain insight into the current use of BRCS, to discuss the clinical practices
2 reported by endodontic practitioners (EPs) and general dental practitioners (GDPs) and to determine if
3 it was in accordance with the best available evidence.
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5 To our knowledge, this is the first international survey addressing BRCS use in root canal obturation.
6 Previously conducted surveys on endodontic practices often included one category of practitioners
7 (either GDPs or EPs) and only within a single country [12–14]. The present survey considered and
8 compared both type of practice and utilized the data-base of a web-based endodontic forum that is
9 available to clinicians world-wide. Despite Endolit being based in the United States, the practitioners
10 registered on this website are from different countries and more than a half of the respondents were
11 not practicing in the United States (55.7%). The number of contacted dentists was consistent (n=2335)
12 and the global response rate achieved (close to 30%) as well as the gender ratio were in line with
13 previously conducted surveys on the dentists' knowledge and type of clinical practice [13,14]. The
14 distribution between EPs and GDPs among respondents (80.3% and 19.7% respectively) was
15 coherent with its distribution in the overall population included in the survey (73.5% of EPs, 26.5% of
16 GDPs).
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18 The results of this survey highlighted a widespread knowledge of BRCS among the respondents
19 (94.8%). Despite the presence of numerous alternative root canal sealers, BRCS was quite popular
20 since 51.7% of the participants used these materials in their clinical practice. Being an EP appeared to
21 be a determining factor in using BRCS ($p<0.05$). The primary reason for using this type of sealers was
22 their improved biological and physicochemical properties (87.7% of BRCS users), regardless of the
23 type of practice. This finding clearly indicates that most practitioners are aware of BRCS properties.
24 Indeed it has been shown that BRCS properties are overall similar or better than those of conventional
25 root canal sealers [9,15]. Indeed, several studies compared physico-chemical and biological properties
26 of different BRCS formulations such as iRoot SP (Innovative Bioceramix, Vancouver, Canada) /
27 EndoSequence BC (Brasseler, Savannah, GA, USA), BioRoot™ RCS (Septodont, Saint-Maur-des-
28 Fossés, France) or Endoseal MTA (Maruchi, Wonju, Korea) versus widely used root canal sealers
29 such as Pulp Canal Sealer (SybronEndo, Orange, CA, USA) a zinc oxide eugenol-based sealer, or AH
30 Plus (Dentsply DeTrey GmbH, Konstanz, Germany) an epoxy resin-based sealer [9,15].
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32 BRCS generally fulfil general endodontic sealer requirements (ISO 6876) regarding radio-
33 opacity, film thickness, solubility and flow [9,15,16]. The specific biological properties of BRCS are due
34 to the presence of calcium silicates whose setting reaction leads to hydration byproducts such as
35 hydroxyl and calcium ions and calcium hydroxide followed by a precipitation reaction with calcium
36 phosphate leading to hydroxyapatite formation. This results in dentinal interaction forming a mineral
37 infiltration zone [17] with higher tubule penetration and push-out strength properties for iRoot SP [15].
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39 BRCS generally provide a good antimicrobial activity [15] without cell toxicity [18,19] and are also
40 reported to be biocompatible inducing periodontal ligament stem cell proliferation and migration [20].
41 Moreover, previous studies have demonstrated BRCS bioactivity on periodontal cells. Indeed their
42 interaction with periodontal ligament cells demonstrated an increased secretion of angiogenic growth
43 factors (VEGF and FGF-2) and the osteogenic factors (BMP-2) [18]. In addition, *in vitro* stimulation
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1 murine osteoblast precursor cell line by BRCS led to an increased DMP-1 expression and mineral
2 deposition [19]. Therefore, even if several factors impact the outcome of root canal treatment these
3 biological properties may help the periodontal healing process in case of apical periodontitis.

4 The attraction toward a “new” product and simplification of the root canal filling procedure appeared as
5 strong arguments in favor of BRCS usage for GDPs ($p<0.05$). However, our findings showed that the
6 clinical practices still remain controversial. For instance, it is well known that drying the canals is a
7 prerequisite before applying conventional endodontic sealers since moisture can affect their setting
8 and adhesion properties [21]. On the contrary, hydration of BRCS is needed to trigger the setting
9 process and to take advantage of their enhanced properties previously mentioned. Surprisingly, our
10 investigation showed that around 10% of BRCS users used alcohol in combination with a paper point
11 to dry the canals, which is known to lead to dentin dehydration [22,23]. The degree to which such
12 procedures can impact the bond strength of BRCS deserves further investigation especially since
13 different adverse effects have been shown between some root canal irrigants and BRCS [24,25].

14 In a similar manner, changes in BRCS physical properties has been shown to occur when subjected to
15 high temperatures associated with warm vertical obturation techniques [10,11]. Despite this fact,
16 almost half of the respondents (46.1%) including more EPs ($p<0.05$) implemented-thermoplasticized
17 gutta-percha obturation techniques. These findings suggest that many practitioners, a fortiori EPs,
18 may tend to rely on their clinical convenience when using BCRS rather than considering the available
19 evidence or respecting the manufacturers’ recommendations. It should be noted that a new BRCS has
20 been recently introduced that is intended to be used with warm gutta-percha techniques (BC HiFlow,
21 Brassler, Savannah, GA, USA) [26]. This survey, however, was conducted prior to the release of this
22 product to the dental market.
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34 In a recently published clinical study, the use of SCT with BRCS has shown a high success rate
35 (around 90%) for both initial treatment and retreatment [27]. However, no long-term clinical studies
36 about BRCS usage are available yet. The presence of voids has been observed regardless of the root
37 canal filling technique and may result in the proliferation of residual microorganisms and jeopardize
38 the long-term outcome [28]. Although SCT may be suitable for filling tapered-rounded canals, it can
39 result in more voids in irregularly shaped anatomies compared to other filling techniques, regardless of
40 the type of the sealer [29–32]. In the present study, 63.3% of BRCS users implemented SCT for root
41 canal obturation in some of their cases and around 40% used it on every case. Among BRCS users,
42 38.4% thought that SCT combined with BRCS can be indicated on every case regardless of the root
43 canal configuration, especially among GDPs ($p<0.05$). EPs, however, tended to adapt the obturation
44 method to the type of sealer. SCT was significantly associated with BRCS usage while warm vertical
45 compaction was the most used obturation technique with sealers other than BRCS ($p<0.05$). Among
46 the participants, 51% declared employing devices such as an injection tip or a Lentulo spiral for sealer
47 insertion into the root canal. Such practices have been shown to reduce voids in the root canal filling
48 [33] and may be recommended.
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57 Sealability of SCT with BRCS partially depends on the interface between the gutta-percha
58 (hydrophobic) and the sealer (hydrophilic). Some manufacturers claim improvement of this interface
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1 thanks to the use of nanoparticles bioceramic pre-impregnated gutta-percha cones. However, a recent
2 study concluded that conventional gutta-percha cones usage showed a slightly better interface with
3 BRCS than pre-impregnated ones [34]. Thus, the fact that only 22% of BRCS users implemented
4 these specific pre-coated gutta cones may not affect the obturation quality.

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6 Finally, one of the controversies linked to BRCS usage is their retreatability since the material may
7 become impenetrable given its hardness upon setting [35]. Most studies have shown that apical
8 patency can be properly re-established if the master cone fitted to the full working length [35–39].
9 However, the adequacy between the retreatment method and the clinical relevance can be questioned
10 since most non-surgical retreatments are indicated in case of obturation short of the radiographic apex
11 and associated with apical periodontitis [40]. While patency has been reported to be achieved when
12 chloroform is used [41], other studies reported that it was often impossible to gain apical patency on
13 curved canals when fillings are short of the working length [35]. This suggests that BRCS presence
14 beyond the master cone may make endodontic retreatment challenging. The fact that 44.4% of BRCS
15 users considered that these sealers may not influence their ability to re-establish apical patency during
16 retreatment (especially among EPs $p < 0.05$) suggests either that BRCS retreatability may not be
17 considered as a major clinical issue or that practitioners are underestimating this problem. **It should
18 also be taken into account that non surgical endodontic retreatment does not only aim to re-establish
19 apical patency but also achieving complete removal of the previous root canal filling materials as well
20 as cleaning and disinfection of the root canal space [42].**

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30 Some limitations should be noted regarding the present survey. Indeed, it utilized a web-based forum
31 primarily dedicated to endodontics. Thus, it can be hypothesized that the GDPs population surveyed
32 may have a particular interest in this specialty. Therefore, our findings should not be generalized to all
33 dentists. As with any survey, another limitation of the study is the self-declaration of the respondents
34 concerning their type of practice. Indeed, the specific requirements to become EPs are location
35 sensitive as well as the recognition of endodontics as an official dental specialty.

40 **Conclusions**

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42 This study highlights wide variation in the clinical practices which are not often in accordance with the
43 current literature on BRCS. The participants of this international survey tend to rely on their clinical
44 habits rather than following the manufacturers' recommendations or considering the best available
45 evidence when using BCRS. This inconsistency implies to provide further clarifications on BRCS
46 clinical application which may pave the way to better standardize their use and improve their future
47 evaluation.
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51 **Acknowledgment**

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56 The authors of this manuscript deny any conflict of interest.

57 **Compliance with ethical standards**

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60 **Conflict of interest:** Dr Maud Guivarc'h declares that she has no conflict of interest. Dr Charlotte
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Jeanneau declares that she has no conflict of interest. Dr Thomas Giraud declares that he has no conflict of interest. Dr Ludovic Pommel declares that he has no conflict of interest. Prof Imad About declares that he has no conflict of interest. Dr Adham A Azim declares that he has no conflict of interest. Prof Frédéric Bukiet declares that he has no conflict of interest.

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Ethical approval: This survey was approved by the Ethics Committee of Aix-Marseille University, France (reference 2018-06-02-002).

Informed consent: Informed consent was obtained from all individual participants included in the study.

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Figures Legends

Figure 1: Survey questionnaire

Figure 2: Obturation techniques implemented by BRCS users with BRCS according to the type of practice (%) (n=349)

CLC, Cold Lateral Compaction; GDPs, General dental practitioners; EPs, Endodontic practitioners; SCT, Single Cone Technique; WV, Warm Vertical. (*) represents significant differences between two groups (p<0.05).

Figure 3: Obturation techniques implemented by BRCS users according to the type of sealer (%) (n=349)

CLC, Cold Lateral Compaction; SCT, Single Cone Technique; WV, Warm Vertical. (*) represents significant differences between two groups (p<0.05).

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Table 1 Distribution of participants (n=675)

Continent	%
<i>Africa</i>	4.2
<i>Asia/Australia</i>	16.3
<i>Europe</i>	22.8
<i>North America</i>	50.8
<i>South America</i>	5.9
Age (years)	
<i><30</i>	12.2
<i>30-39</i>	31.0
<i>40-49</i>	24.4
<i>50-59</i>	15.7
<i>≥60</i>	16.7
Gender	
<i>Male</i>	72.3
<i>Female</i>	27.7
Years of Practice	
<i>0-10</i>	34.5
<i>11-20</i>	29.3
<i>21-30</i>	16.9
<i>≥30</i>	19.3
Type of practice	
<i>GDPs</i>	19.7
<i>EPs</i>	80.3

Table 2 Knowledge and usage of BRCS

	GDPs (%)	Eps (%)	Global (%)	<i>P</i> Value
Has already heard of BRCS (n=675)	83.5	97.6	94.8	p<0.05
Has already used BRCS (n=640)	41.0	62.0	58.4	p<0.05
↳ From “sometimes” to “always” (n=374) (<i>BRCS users</i>)	95.7	93.0	93.3	p>0.05

Table 3 Reasons for using BRCS (n=349)

	GDPs (%)	EPs (%)	Global (%)	<i>P</i> Value
Biological and physicochemical properties	88.6	87.5	87.7	p>0.05
Possibility to fill simultaneously the root canal and a perforation	38.6	36.4	36.7	p>0.05
Attraction toward a new biomaterial	47.7	30.2	32.4	p<0.05
Simplification and saving time	47.7	23.9	26.9	p<0.05
Connection with minimal invasive dentistry	29.5	14.4	16.3	p<0.05
No specific equipment for obturation	36.4	12.1	15.8	p<0.05

Questionnaire

Dear colleagues,

We would like to attract your attention on an online study regarding your practice in root canal filling. We would be very grateful if you could give us some of your time by spending a few minutes filling out a questionnaire. Filling the questionnaire does not require any specific knowledge and only refers to your own clinical experience. The time required is about 5 minutes and the data collected is strictly anonymous. There is absolutely no obligation to participate in this study and your decision to contribute will be considered as informed consent. Aware that your time is valuable, we are very grateful for the attention you will give to the completion of this survey.

Thank you for your time and assistance.

1. I have received information about the conditions of this study and I give my informed consent to contribute.

- Yes No (end of the questionnaire)

2. Have you ever heard about Bioceramic root canal sealers (BRCS)?

- Yes No (go to question 13)

3. How have you first known about BRCS?

- Through conferences / courses Through manufacturers / advertising
 Through another dentist's experience Other

4. Have you ever used BRCS?

- Yes No (go to question 13)

5. At what frequency do you estimate the use of BRCS in your daily practice?

- Never (go to question 13) Sometimes
 Frequently Always (i.e. in every case)

6. When using BRCS, how do you generally dry the root canal?

- Sterile paper points alone With alcohol before using sterile paper points
 With sterile paper points combined with Luer vacuum adapter (or equivalent system)
 Other (specify)

7. When using BRCS, how do you generally apply the sealer?

- Simple gutta cone coating Injection tip
 Lentulo spiral Other (specify)

8. When using BRCS, which obturation technique do you use? (multiple answers possible)

- Warm vertical compaction Cold lateral compaction Carrier-based obturation
 Single cone technique Other (specify)

9. What type of gutta percha cones do you currently use with BRCS?

- Bioceramic nanoparticles pre-coated gutta percha cones Non pre-coated cones

10. What was your motivation to use BRCS in your practice? (multiple answers possible)

- Biological and physico-chemical properties of this type of sealers
 Significant saving time and simplification
 No need of special equipment devoted to root canal obturation
 Possibility to fill simultaneously the root canal and a root perforation
 Connection with minimal invasive endodontics (preservation of coronal, pericervical and radicular dental tissue)

Willingness to try this recent biomaterial

11. It is generally recommended to use BRCS with single cone technique. Do you think this method can be indicated regardless of the root canal anatomy?

Yes No

12. Do you think BRCS usage may influence the ability to re-establish apical patency during non-surgical retreatment?

Yes No

13. If you do not use BRCS, which obturation technique do you use? (multiple answers possible)

Warm vertical compaction Cold lateral compaction Carrier-based obturation
 Single cone technique Other (specify)

14. What is your gender?

Male Female

15. How old are you?

..... (years)

16. In which year did you graduate as a dentist?

.....

17. Do you practice as...

A general practitioner An endodontist practitioner

A postgraduate student/resident in endodontics

18. Where do you practice?

Africa Asia/Australia Europe North America South America

Figure 2

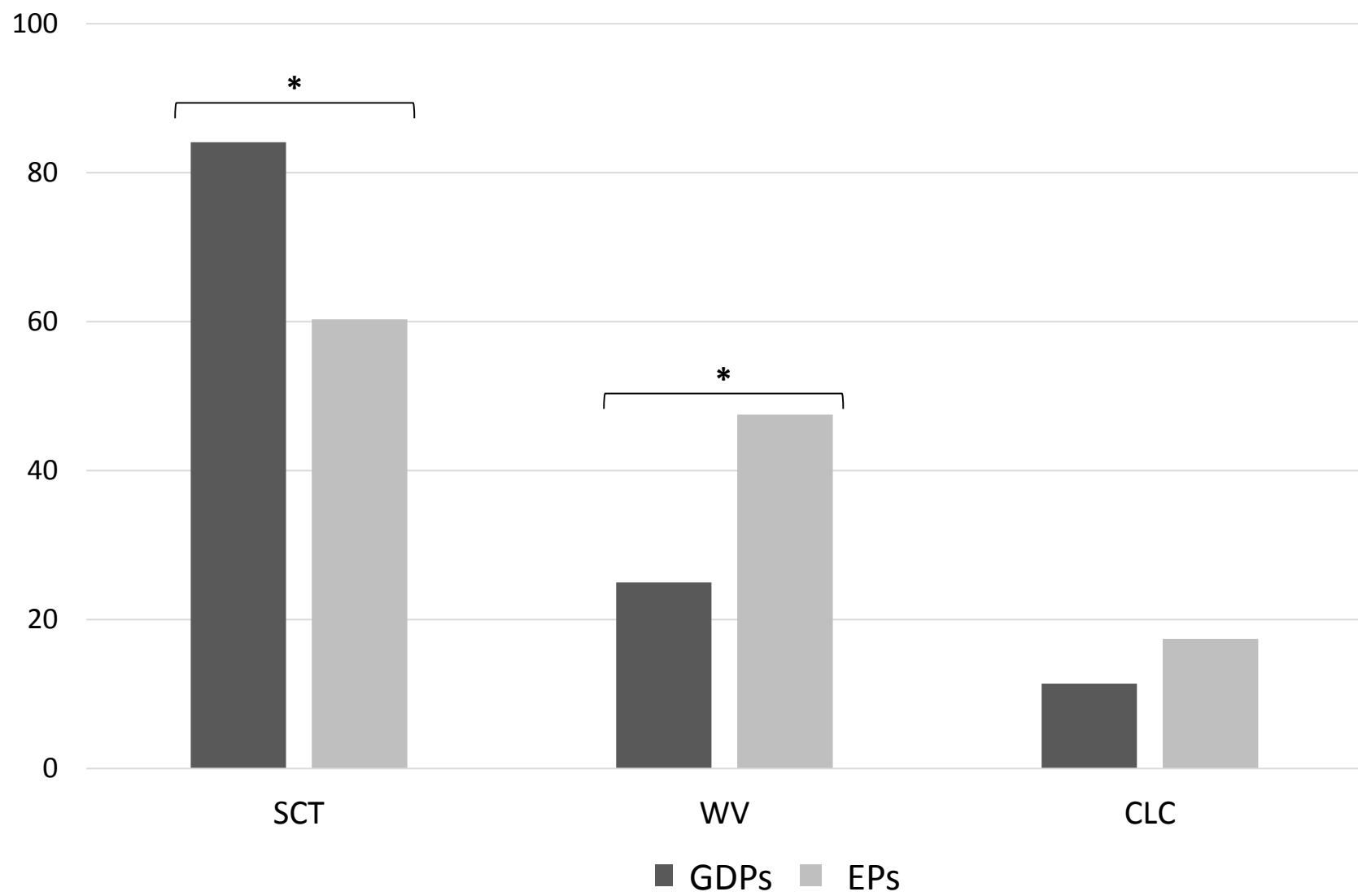


Figure 3

