Unprepared root canal surface areas: causes, clinical implications, and therapeutic strategies

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Abstract: Chemomechanical preparation is intended to clean, disinfect, and shape the root canal. This step is of utmost importance during treatment of infected teeth with apical periodontitis, because treatment outcome depends on how effectively the clinician eliminates bacteria, their products, and necrotic tissue that would serve as substrate for bacterial regrowth. Nonetheless, curvatures and complex internal anatomical variations of the root canal system can pose a high degree of difficulty in reaching these goals. In infected teeth, bacteria may persist not only in difficult-to-reach areas such as isthmuses, ramifications, dentinal tubules, and recesses from C-shaped or oval/flattened canals, but also in areas of the main canal wall that remain untouched by instruments. If bacteria withstand chemomechanical procedures, there is an augmented risk for post-treatment apical periodontitis. This article discloses the reasons why some areas remain unprepared by instruments and discusses strategies to circumvent this issue and enhance infection control during endodontic treatment/retreatment of teeth with apical periodontitis.

Keywords: Root Canal Preparation; Root Canal Therapy; Treatment Outcome; Periapical Periodontitis.

Introduction

Apical periodontitis is a disease of infectious etiology. Consequently, its successful treatment relies upon effective infection control. Chemomechanical preparation can be considered the most important phase of endodontic treatment, because, in addition to carving a shape for proper filling, it eliminates the cause of apical periodontitis – bacterial infection of the root canal. By mechanical and chemical means, bacteria and their products are eliminated from the canal, and necrotic tissue is removed, which might serve as substrate for bacterial regrowth. Healing of apical periodontitis is certainly more predictable when the intracanal bacterial burden is eradicated or at least substantially reduced (disinfection) to levels that are compatible with periradicular tissue repair.

The main risk factor for post-treatment apical periodontitis is the poor quality of previous endodontic treatment. In most cases, technical deficiencies during chemomechanical procedures will result in insufficient cleaning and disinfection, leading to bacterial persistence in the canal.

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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Levels of bacteria in poorly treated canals are expected to be sufficient to sustain periapical inflammation. However, although at a lower frequency, bacteria may persist even when treatment has followed acceptable standards. This occurs because the complexity of the root canal anatomy may pose significant challenges for attainment of proper cleaning, disinfection, and shaping, even for the experienced clinician. Anatomical complexities that may influence the results of chemomechanical procedures include curvatures, isthmuses, lateral canals, apical ramifications, and recesses from oval-shaped, C-shaped, or flattened root canals. Instruments act mainly on the main canal and proper cleaning and disinfection of isthmuses, recesses, and ramifications depend mostly on the chemical effects of irrigants and interappointment medicaments.

Even in the main canal, instruments have been shown not to reach all areas of the canal walls (Figure 1). This happens because of factors related to instrument features and/or canal anatomy. For instance, preparation based on final instruments that are smaller than the initial canal diameter is expected to leave more areas untouched. As for anatomy, a curvature introduces a complexity for instrumentation because the cutting action of the instruments along the curved canal is concentrated more on some walls than on others. Consequently, some areas may remain untouched by the instruments. In addition, C-shaped and oval/flattened canals have an irregular morphology that is inconsistent with round preparations provided by rotary instrumentation systems (Figure 2). Therefore, unsurprisingly, canals with these morphologies exhibit a large amount of uninstrumented areas following preparation, as the instruments may not reach all recesses (Figure 2).

In small and/or round canals, micro-computed tomographic (micro-CT) studies have reported that different instrumentation systems leave approximately 10% to 50% of the full canal surface area unprepared (Figure 3) (Table 1). These figures can be even higher when only the apical canal surface is evaluated (Table 1). As for oval/flattened canals, the amount of untouched surface area after preparation has been shown to range from 10% to 80% (Table 2). This article discusses what happens to areas that remain unprepared, the implications of bacteria remaining in these areas, and strategies to improve root canal cleaning, shaping, and disinfection.

**Antimicrobial effects of preparation procedures**

Because bacterial elimination from the root canal has been regarded as a surrogate endpoint for treatment outcome, numerous studies have evaluated the antimicrobial efficacy of different instrumentation techniques and irrigation substances. Culture-dependent and culture-independent studies have shown that a substantial reduction in bacterial counts is obtained after chemomechanical preparation. The importance of using an antimicrobial substance such as sodium hypochlorite (NaOCl) to supplement the mechanical effects of instrumentation and enhance root canal disinfection has been demonstrated by clinical and in vitro studies. Preparation using 2.5% NaOCl as the irrigant can reduce bacterial levels 10² to 10⁵-fold, with an overall reduction of 95% to 99%. The best NaOCl antimicrobial effects are observed when the substance is regularly exchanged and used in large volumes. Chlorhexidine (CHX) has also been used for irrigation as an alternative to NaOCl and has shown similar antimicrobial results.

Notwithstanding the pronounced bacterial reduction promoted by NaOCl or CHX in association with mechanical instrumentation, bacteria may still be detected in about 30% to 60% of the canals of teeth with apical periodontitis after preparation with these solutions. It is salient to point out that samples in most studies of this nature are taken with paper points placed in the main canal. Although bacteria located in areas in the immediate vicinity of the main canal can be sampled, the results from these studies are mostly related to the bacteriological conditions of the main canal lumen and walls.

The main question is: how can bacteria manage to avoid the mechanical effects of instruments and irrigation as well as the chemical effects of irrigants, even in the main canal? Mechanical debridement is a powerful means to remove biofilms from any surface. In infected canals, biofilms will only have chances to
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remain if they occur in areas where the instruments do not exert their cutting action and irrigants fail to reach at an effective concentration and/or time. Thus, residual bacterial biofilms located in these areas would remain unaffected or be only partially eliminated, especially if they are thick (Figure 4).

Inefficiency of instruments and irrigants can be a result of inappropriate treatment. For instance, when apical preparation size is too small, large amounts of bacteria and necrotic tissue can be left behind. The same is expected when preparation is too short of the apex, leaving a large extent of unprepared main canal. Poor irrigation due to small volume of irrigant or shallow depth of needle penetration may also compromise cleaning and disinfection.

Figure 1. Extracted mandibular incisor subjected to ex vivo root canal instrumentation. (A) Cross section cut at the middle third. Only a fraction of the canal circumference was shaped by rotary instruments. The canal lumen appears free from soft tissue remnants (hematoxylin and eosin, original magnification ×16). (B) Detail from (A) (original magnification ×50). (C) Longitudinal section of the apical third, encompassing the apical canal and a large apical ramification (original magnification ×16). (D) Middle magnification of the canal area indicated by the arrow in (C). A large mass of pulp tissue remnants can be appreciated. Note that predentin is still present on the canal wall, confirming that the area was not touched by the instruments (original magnification ×100).
As mentioned previously, there is an increased risk of root canal surface areas remaining unprepared if the canal is curved. Moreover, the use of reaming motion for preparation of oval/flattened or C-shaped canals often results in suboptimal cleaning and disinfection.17,73,74,75,76,77 Recesses that remain untouched by instruments may harbor residual biofilms and be packed with infected dentin debris (Figure 4).79,80,81

Evaluating canal preparation with correlative approaches

Root canal cleaning, shaping, and disinfection during preparation have been evaluated by numerous analytical approaches, depending on the parameter under assessment. For instance, cleaning studies have used histology,9,10,82,83 histobacteriology,8 histological evaluation with scanning electron microscopy,84,85 and confocal laser scanning microscopy.86,87

Figure 2. Mandibular incisors with advanced periodontal disease. Tooth 41 had indication for extraction and before that, with the patient’s agreement, its root canal was instrumented with rotary NiTi instruments. (A) Preoperative radiograph. (B) Cross-cut section from the middle third of tooth 41. The walls of the long oval canal were instrumented only on one side, to a minimal extent. Soft tissues are absent (haematoxylin and eosin, original magnification ×16). (C) Magnification of the lingual extension of the canal, indicated by the arrow in (B). Predentin is still present, indicating that instruments had not reached this area, with a minimal amount of soft tissue debris (original magnification ×100). (D) Cross section cut at the transition from middle to apical third. NiTi instruments shaped only one portion of the canal, while the majority of the canal walls appeared untouched, with the lumen filled with pulp tissue remnants (original magnification ×16). (E) Detail from (D) (original magnification ×50). (F) High power view of the area of the canal indicated by the right arrow in (E). Dentin filings enmeshed in a necrotic pulp tissue (original magnification ×400). (G) High power view of the area of the canal indicated by the left arrow in (E). Vital pulp tissue is present at some distance from the instrumented portion of the canal (original magnification ×400).
Shaping studies have made use of radiographs,\textsuperscript{86,87} resin blocks,\textsuperscript{88,89} cone-beam computed tomography,\textsuperscript{90} and micro-computed tomography (micro-CT).\textsuperscript{7,25} Studies evaluating disinfection have used histology/\textit{transmission} electron microscopy,\textsuperscript{91} histobacteriology,\textsuperscript{8} culture,\textsuperscript{51,64,92} and molecular methods.\textsuperscript{52,55,93} Findings presented by these methods have been successfully used to elucidate the effects of chemomechanical procedures and improve instruments, irrigants, and techniques over the years.

Over the last decade, micro-CT has been certainly the most widely used methodology to investigate the shaping effects of instruments and instrumentation techniques. Its wide use and acceptance mostly refers to its nondestructive nature, which permits one to compare the root canal morphology before and after preparation using extracted teeth. Overlapping of

![Superimposed micro-computed tomographic images](image)

**Figure 3.** Superimposed micro-computed tomographic images taken before (green) and after (red) the use of rotary NiTi instruments revealing areas that remained unprepared (green).

<table>
<thead>
<tr>
<th>Study</th>
<th>Tooth type</th>
<th>Instrument</th>
<th>Area evaluated (full or apical canal)</th>
<th>Uninstrumented area (%)</th>
<th>Statistical findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peters et al.</td>
<td>Max M</td>
<td>GT</td>
<td>Full canal</td>
<td>43%</td>
<td>No significant differences</td>
</tr>
<tr>
<td>(2001)\textsuperscript{17}</td>
<td></td>
<td>Lightspeed</td>
<td>Full canal</td>
<td>37.5%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NiTi Hand Files</td>
<td>Full canal</td>
<td>35%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Profile</td>
<td>Full canal</td>
<td>37%</td>
<td></td>
</tr>
<tr>
<td>Peters et al.</td>
<td>Max M</td>
<td>K-File</td>
<td>Full canal</td>
<td>53.5%</td>
<td>No significant differences</td>
</tr>
<tr>
<td>(2001)\textsuperscript{13}</td>
<td></td>
<td>Lightspeed</td>
<td>Full canal</td>
<td>39%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Profile</td>
<td>Full canal</td>
<td>43%</td>
<td></td>
</tr>
<tr>
<td>Peters et al.</td>
<td>Max M MB</td>
<td>PT</td>
<td>Full canal</td>
<td>43%</td>
<td>No significant differences</td>
</tr>
<tr>
<td>(2003)\textsuperscript{22}</td>
<td>Max M DB</td>
<td>PT</td>
<td>Full canal</td>
<td>33%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max M P</td>
<td>PT</td>
<td>Full canal</td>
<td>49%</td>
<td></td>
</tr>
<tr>
<td>Paqué et al.</td>
<td>Max M MB</td>
<td>Endo-Eze</td>
<td>Full canal</td>
<td>30%</td>
<td>No significant differences</td>
</tr>
<tr>
<td>(2005)\textsuperscript{33}</td>
<td>Max M DB</td>
<td>Endo-Eze</td>
<td>Full canal</td>
<td>25%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max M P</td>
<td>Endo-Eze</td>
<td>Full canal</td>
<td>29%</td>
<td></td>
</tr>
<tr>
<td>Paqué et al.</td>
<td>Max M</td>
<td>Flexmaster</td>
<td>Full canal/apical canal</td>
<td>~43%/~42%</td>
<td>GT left significantly more untreated areas</td>
</tr>
<tr>
<td>(2009)\textsuperscript{14}</td>
<td></td>
<td>GT</td>
<td>Full canal/apical canal</td>
<td>~44%/~71%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lightspeed</td>
<td>Full canal/apical canal</td>
<td>~42%/~50%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NiTi Hand Files</td>
<td>Full canal/apical canal</td>
<td>~40%/~50%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Profile</td>
<td>Full canal/apical canal</td>
<td>~42%/~45%</td>
<td></td>
</tr>
<tr>
<td>Paqué et al.</td>
<td>Mand M IV</td>
<td>PT F2 reciprocating</td>
<td>Full canal/apical canal</td>
<td>16%/25%</td>
<td>No significant differences</td>
</tr>
<tr>
<td>(2011)\textsuperscript{25}</td>
<td></td>
<td>PT Full sequence</td>
<td>Full canal/apical canal</td>
<td>19%/30%</td>
<td></td>
</tr>
<tr>
<td>Peters and Paqué (2011)</td>
<td>Max M MB</td>
<td>SAF</td>
<td>Full canal/apical canal</td>
<td>26%/NS</td>
<td>No significant differences</td>
</tr>
<tr>
<td></td>
<td>Max M DB</td>
<td>SAF</td>
<td>Full canal/apical canal</td>
<td>22%/29%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max M P</td>
<td>SAF</td>
<td>Full canal/apical canal</td>
<td>25%/47%</td>
<td></td>
</tr>
</tbody>
</table>

Continue
<table>
<thead>
<tr>
<th>Authors</th>
<th>Study Type</th>
<th>Max M/ Mand M</th>
<th>Instrument</th>
<th>Canal Type</th>
<th>Success Rate</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang et al. (2011)</td>
<td></td>
<td>Mand M IV</td>
<td>Mtwo, PTU</td>
<td>Full canal</td>
<td>42%</td>
<td>No significant differences</td>
</tr>
<tr>
<td>Markvart et al. (2012)</td>
<td></td>
<td>Max/Mand M</td>
<td>GT/Profile</td>
<td>Full canal, coronal canal/middle canal/ apical canal</td>
<td>38%/32%/45%/39%</td>
<td>No significant differences</td>
</tr>
<tr>
<td>Siqueira et al. (2013)</td>
<td></td>
<td>Mand M IV</td>
<td>Reciproc, SAF, TF</td>
<td>Full canal</td>
<td>21%</td>
<td>No significant differences</td>
</tr>
<tr>
<td>Zhao et al. (2013)</td>
<td></td>
<td>Max M</td>
<td>Hylex CM, K3, TF</td>
<td>NS</td>
<td>41%</td>
<td>No significant differences</td>
</tr>
<tr>
<td>Gergi et al. (2015)</td>
<td></td>
<td>Mand M IV</td>
<td>Reciproc, TFA, WaveOne</td>
<td>Full canal, apical canal</td>
<td>20.5%/25%</td>
<td>Reciproc left significantly less untreated areas</td>
</tr>
<tr>
<td>Zhao et al. (2014)</td>
<td></td>
<td>Mand M IV</td>
<td>PTN, PTU, WaveOne</td>
<td>NS</td>
<td>41.5%; 37%; 55%</td>
<td>No significant differences</td>
</tr>
<tr>
<td>De-Deus et al. (2015)</td>
<td></td>
<td>Mand M II</td>
<td>BR3, BR5, Reciproc 25/08; 40/06, WaveOne 25/08; 40/08</td>
<td>Full canal</td>
<td><del>45</del>/~41</td>
<td>Significant reduction in the percentage of untreated areas after larger preparation</td>
</tr>
<tr>
<td>Gagliardi et al. (2015)</td>
<td></td>
<td>Mand M IV</td>
<td>PTG, PTN, PTU</td>
<td>Full canal</td>
<td>3.5%</td>
<td>PTN showed significantly more untreated areas</td>
</tr>
<tr>
<td>Peters et al. (2015)</td>
<td></td>
<td>Mand M IV</td>
<td>TruShape 20/06v; 30/06v, Vortex Blue 20/06v; 30/06</td>
<td>Full canal, apical canal</td>
<td>28.5% 15%/43.5%; 19%</td>
<td>Enlargement to size #30 significantly increased the amount of prepared area</td>
</tr>
<tr>
<td>Alves et al. (2016)</td>
<td></td>
<td>Mand M IV</td>
<td>BR + PUI, BR + Xp Endo Finisher</td>
<td>Full canal</td>
<td>4%</td>
<td>No significant differences</td>
</tr>
<tr>
<td>Limoeiro et al. (2016)</td>
<td></td>
<td>Mand M IV</td>
<td>BR, PTN</td>
<td>Full canal continuing to size #30</td>
<td>12%</td>
<td>No significant differences</td>
</tr>
<tr>
<td>Brasil et al. (2017)</td>
<td></td>
<td>Mand M IV</td>
<td>BTR, PTN</td>
<td>Full canal, apical canal</td>
<td>33%/14%</td>
<td>No significant differences</td>
</tr>
<tr>
<td>Duque et al. (2017)</td>
<td></td>
<td>Mand M IV</td>
<td>PTG F2/F3, PTU F2/F3</td>
<td>Full canal, apical canal</td>
<td>10%/6%</td>
<td>Significant reduction in the percentage of untreated areas after larger preparation</td>
</tr>
<tr>
<td>Siqueira et al. (2018)</td>
<td></td>
<td>Mand M IV</td>
<td>Reciproc</td>
<td>Full canal, apical canal</td>
<td>18%/10%</td>
<td>NC</td>
</tr>
</tbody>
</table>

Max M: maxillary molars; Max M IV: Maxillary molars mesiobuccal canals Vertucci's type IV; Mand M IV: Mandibular molars mesial canals Vertucci’s type IV; Mand M II: Mandibular molars mesial canals Vertucci’s type II; MB: Mesiobuccal canal; DB: Disto-buccal canal; ML: Mesiolingual canal; D: Distoal canal; P: Palatal canal; NS: Not specified; NC: No intergroup/intragroup comparison; BR: BioRaCe; BTR: BT-Race; GT: Greater taper; PT: ProTaper; PTG: ProTaper Gold; PTN: ProTaper Next; PTU: ProTaper Universal; SAF: Self-Adjusting File; TF: Twisted file; TFA: Twisted File Adaptive
images taken before and after the use of instruments reveals areas that remained uninstrumented (Figure 3). Numerous studies have shown that an overall large surface area of the canal remains unprepared, regardless of the instrument used, and this is still more critical in curved and/or oval/flattened canals.\textsuperscript{16,22,23,24}

As discussed above, uninstrumented areas may potentially harbor remnants of bacterial biofilms.\textsuperscript{16,27} One might assume that even if the instruments fail to reach all canal walls, these areas would be permeated by NaOCl used for irrigation, which could dissolve necrotic tissue remnants and kill residual bacteria. In this case, the irrigant should reach the uninstrumented areas at an effective concentration and volume and remain therein long enough for it to take effect.

Studies have evaluated only one parameter at a time, \textit{i.e.}, either the amount of untouched walls or the cleaning/disinfection effects of preparation. Recently, several studies have been conducted by our group to investigate what happens to the uninstrumented surface areas by using a correlation between micro-CT and another analytical method for cleaning or disinfection (Figure 5).

In the first study,\textsuperscript{16} we combined findings from micro-CT and microbiological methods for a correlative evaluation of the effects of canal preparation using different instrumentation systems. Findings revealed no correlation between the amount of unprepared surface areas and residual bacterial levels.
Table 2. Amount of uninstrumented areas after preparation of oval canals. Studies using micro-computed tomography

<table>
<thead>
<tr>
<th>Study</th>
<th>Tooth type</th>
<th>Instrument</th>
<th>Area evaluated (full or apical canal)</th>
<th>Uninstrumented area (%)</th>
<th>Statistical findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paqué et al. (2010)17</td>
<td>Mand M D</td>
<td>PT1</td>
<td>Full canal/apical canal</td>
<td>80%/69%</td>
<td>PT1 left significantly more untreated areas than PT2 in the full canal</td>
</tr>
<tr>
<td></td>
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<td>PT2</td>
<td>Full canal/apical canal</td>
<td>60%/65%</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>PT-CF</td>
<td>Full canal/apical canal</td>
<td>NS/65%</td>
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<tr>
<td></td>
<td></td>
<td>H-CF</td>
<td>Full canal/apical canal</td>
<td>NS/75%</td>
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<tr>
<td>Paqué and Peters (2011)12</td>
<td>Mand M D</td>
<td>SAF 1.5 mm</td>
<td>Full canal/apical canal</td>
<td>23%/40%</td>
<td>SAF resulted in less untreated surface for both full and apical canal</td>
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<tr>
<td></td>
<td></td>
<td>PT-2</td>
<td>Full canal/apical canal</td>
<td>60%/65%</td>
<td></td>
</tr>
<tr>
<td>Peters and Paqué (2011)43</td>
<td>Max M MB</td>
<td>SAF 1.5 mm</td>
<td>Full canal/apical canal</td>
<td>26%/NS</td>
<td>No significant differences</td>
</tr>
<tr>
<td>Versiani et al. (2011)40</td>
<td>Mand Inc</td>
<td>K3</td>
<td>Coronal/middle/apical</td>
<td>38%/56%/25%</td>
<td>K3 left significantly more untreated areas than SAF in coronal and middle thirds</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SAF 1.5 mm</td>
<td>Coronal/middle/apical</td>
<td>8%/35%/15%</td>
<td></td>
</tr>
<tr>
<td>Zhao et al. (2014)37</td>
<td>Mand M IV</td>
<td>WaveOne</td>
<td>Full canal MB/ML</td>
<td>40%/35%</td>
<td>No significant differences</td>
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<td></td>
<td></td>
<td>PTN</td>
<td>Full canal MB/ML</td>
<td>41.5%/37%</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>PTU</td>
<td>Full canal MB/ML</td>
<td>41%/38%</td>
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<td>Busquim et al. (2015)39</td>
<td>Mand M D</td>
<td>BR</td>
<td>Full canal/coronal/middle/apical</td>
<td>10%/8%/11%/10%</td>
<td>Reciproc left significantly more untreated areas than BR5 in full, coronal and middle canal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reciproc</td>
<td>Full canal/coronal/middle/apical</td>
<td>15%/18%/22%/5%</td>
<td>SS left significantly more untreated areas than PT in all thirds</td>
</tr>
<tr>
<td>Stavileci et al. (2015)43</td>
<td>Max PM</td>
<td>PT</td>
<td>Coronal/middle/apical</td>
<td>26%/35%/20%</td>
<td>No significant differences</td>
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<td></td>
<td></td>
<td>SS</td>
<td>Coronal/middle/apical</td>
<td>29%/41%/25%</td>
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<tr>
<td>Coelho et al. (2016)44</td>
<td>Mand Inc</td>
<td>Easy Prodesign</td>
<td>Coronal/middle/apical</td>
<td>64%/62%/70%</td>
<td>No significant differences</td>
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<td></td>
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<td>OneShape</td>
<td>Coronal/middle/apical</td>
<td>79%/72%/75%</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>WaveOne</td>
<td>Coronal/middle/apical</td>
<td>80%/77%/75%</td>
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<tr>
<td>Arias et al. (2017)46</td>
<td>Mand M D</td>
<td>TRUSHape</td>
<td>Full canal/apical canal</td>
<td>56%/50%</td>
<td>No significant differences</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vortex Blue</td>
<td>Full canal/apical canal</td>
<td>51%/51%</td>
<td></td>
</tr>
<tr>
<td>Espir et al. (2017)46</td>
<td>Mand Inc</td>
<td>Mtwo</td>
<td>Full canal/coronal/middle/apical</td>
<td>17%/5%/7%/4%</td>
<td>No significant differences</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reciproc</td>
<td>Full canal/coronal/middle/apical</td>
<td>18%/3%/6%/4%</td>
<td></td>
</tr>
<tr>
<td>Guimarães et al. (2017)45</td>
<td>Mand PM</td>
<td>Reciproc</td>
<td>Full canal/apical canal</td>
<td>24%/25%</td>
<td>In the full canal Reciproc left significantly more untreated areas than TRUSHape</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TRUSHape</td>
<td>Full canal/apical canal</td>
<td>30%/20%</td>
<td></td>
</tr>
<tr>
<td>Lacerda et al. (2017)49</td>
<td>Mand M D</td>
<td>SAF</td>
<td>Full canal/apical canal</td>
<td>11%/10%</td>
<td>No significant differences with TRUSHape</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TRUSHape</td>
<td>Full canal/apical canal</td>
<td>17.5%/16%</td>
<td>No significant differences in the full canal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Xp Endo Shaper</td>
<td>Full canal/apical canal</td>
<td>17%/18%</td>
<td>BR left significantly more untreated areas than Reciproc and SAF</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BR</td>
<td>NS</td>
<td>32%</td>
<td>No significant differences with TRUSHape</td>
</tr>
<tr>
<td>Zuolo et al. (2017)47</td>
<td>Mand Inc</td>
<td>Reciproc</td>
<td>NS</td>
<td>19%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SAF</td>
<td>NS</td>
<td>16%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TRUSHape</td>
<td>NS</td>
<td>19%</td>
<td></td>
</tr>
</tbody>
</table>

Max M: Maxillary molars; Max M MB: Maxillary molars mesiobuccal canal; Max M MB MB: Maxillary premolars Mand M D: Mandibular molars distal canal; Mand Inc: Mandibular incisors; Mand PM: Mandibular premolars; Mand M IV: Mandibular molars mesial canals Vertucci’s type IV; NS: Not specified; BR: BioRaCe; CF: Circumferential filing; GT: Greater Taper; H: Hedström files; PT: ProTaper; PTG: ProTaper Gold; PTN: ProTaper Next; PTU: ProTaper Universal; SAF: Self-Adjusting File; SS: Stainless-steel hand files
In another micro-CT/microbiology correlative study, Alves et al.\textsuperscript{30} evaluated the supplementary disinfecting ability of passive ultrasonic irrigation (PUI) and XP-endo Finisher in the mesial canals of mandibular molars. Teeth were anatomically matched between groups based on micro-CT parameters, the canals were contaminated, instrumented using NaOCl irrigation, and the supplementary approaches were used. Samples were taken from the main canal and from the isthmus area identified by micro-CT and processed by cryopulverization. Quantitative polymerase chain reaction analysis demonstrated that both supplementary approaches resulted in a small additional bacterial reduction, which was significant only for XP-Endo Finisher. Confirming the previous study\textsuperscript{16}, correlative analysis revealed no relationship between the amount of unprepared surface area and residual bacterial counts. Molecular microbiology analysis of the cryopulverized root fragments corresponding to the isthmus area showed no effective disinfection with either approach.\textsuperscript{30}

A correlative study using micro-CT and histology or SEM evaluated what happens to canal walls of necrotic and vital teeth that remained uninstrumented following preparation with a reciprocating instrument and NaOCl irrigation.\textsuperscript{31} The unprepared surface areas were identified by micro-CT and their morphological conditions were examined microscopically. Overall, in the vast majority of specimens, the unprepared areas were covered with bacteria and/or remnants of pulp tissue.

Another recent study evaluated the cleaning and shaping ability of three instrumentation systems in oval canals using a correlative micro-CT/histological approach.\textsuperscript{49} Canals matched by anatomic similarities were prepared by using SAF, TRUShape, and XP-endo Shaper instruments, with 5.25% NaOCl irrigation. No significant difference was found for the amount of unprepared surface areas between the three instrument systems, except when comparing SAF and XP-endo Shaper in the apical canal (the former was better). No difference was found in the cleaning ability either, with about half of the specimens still exhibiting pulp remnants in some uninstrumented buccal and lingual recesses.

These correlative studies demonstrated that unprepared canal surfaces and isthmuses are not predictably cleaned and disinfected by conventional irrigation with NaOCl. The ability of bacteria to persist in these areas and influence the treatment outcome depends on some factors.

**When bacteria persisting in unprepared areas influence the treatment outcome**

Persistent intraradicular infection is the most common cause of post-treatment apical periodontitis.\textsuperscript{2} For bacteria to withstand the effects of root canal treatment, they need to resist intracanal disinfection procedures, and adapt to the drastically changed environment. Although some bacteria may be resistant to some antimicrobial substances used in endodontics,\textsuperscript{94,95} the main reason for bacterial persistence is the inability of current techniques and medications to disinfect the entire extent of the root canal system.

In addition to enduring antimicrobial treatment, residual bacteria need to adapt to the drastically changed environment in order to survive and sustain periradicular inflammation.\textsuperscript{96} The whole ecology of the endodontic microenvironment is altered by treatment, so the surviving bacteria need to adapt to the new conditions.

Finding a source of nutrients is an essential prerequisite for bacterial survival. Remaining bacteria may utilize necrotic tissue remnants in untouched areas, recesses, isthmuses, and lateral canals as substrate, which is, however, a finite source. A sustainable nutrient source should exist and may develop as tissue fluids and inflammatory exudates from the periradicular tissues seep into the canal as a consequence of an inappropriate apical seal or a too short apical preparation/obturation.\textsuperscript{96} In most teeth with post-treatment disease, bacteria are located in the apical part of the root canal system, usually in the main canal lumen interposed between the filling material and the periapical tissues, in unprepared and unsealed areas and/or within apical ramifications.\textsuperscript{97,98,99} In these locations, residual bacteria have easy access to nutrients.
Persistent bacteria influence the endodontic treatment outcome when:

a. they withstand periods of nutrient deprivation immediately after chemomechanical procedures, scavenging for traces of nutrients and/or assuming a dormant state, to prosper again when a sustainable nutrient source is re-established;

b. they adapt to the new ecological conditions of the canal, including oxygen tension, types of nutrient available, and co-existing species, all changed by treatment procedures;

c. they reach critical numbers to cause damage and stimulate inflammation in the periradicular tissues;

d. they have unrestrained access to the periradicular tissues through apical/lateral foramina or iatrogenic perforations;

e. they release virulence factors that are expressed in the modified environment and reach sufficiently high concentrations to sustain inflammation.

Future challenge – reducing unprepared areas and/or improving disinfection

Morphological studies of teeth with post-treatment apical periodontitis (endodontic treatment failure) have reported the occurrence of persistent bacterial infection in isthmuses, lateral canals and apical ramifications, recesses, and dentinal tubules. Current instrumentation systems fail to predictably touch all canal walls and reach these areas distant from the main canal. Therefore, there is an urgent need to develop strategies to improve infection control not only in the main canal lumen, but also in the entire root canal system.

Apparently, a logical strategy to reduce the amount of unprepared areas is to increase the size of apical preparation. Chemomechanical procedures should be considered complete when the canal is enlarged to instrument sizes that are compatible with the root anatomy and size, and sufficiently large to be safe and antibacterially effective. A micro-CT

Figure 5. (A) Representative 3D reconstruction of micro-computed tomographic scans taken before (green) and after (red) root canal preparation with a rotary NiTi instrument showing unprepared areas (green). (B) Representative cross-section of the middle third region marked in (A), showing an unprepared recess (arrow). (C) Correlative histological section revealing pulp tissue remnants in the recess (arrow).
study demonstrated that after each increase in apical preparation size, the amount of unprepared surfaces reduced significantly. Microbiological studies have revealed that the larger the apical preparation size of infected canals, the greater the intracanal bacterial reduction. Studies have also shown that the larger the apical preparation size, the cleaner the apical canal. Larger preparations are also associated with improved outcome of the treatment of infected teeth with apical periodontitis.

It is salient to point out that the width of canal preparation should be large enough in the apical portion to optimize disinfection and cleaning and at the same time not be too large so as not to cause accidents (ledges or perforation) or weaken the root. Overenlargement of the coronal part of the canal is undesirable, in order not to predispose to fracture. Instruments with variable tapers have been introduced (Reciproc, WaveOne, ProTaper, etc) and help deal with this issue, i.e., they permit large apical preparations, reducing unnecessary coronal enlargement.

Another strategy that has been widely recommended is to supplement the effects of chemomechanical procedures and enhance disinfection. Application of an interappointment medicament has been consistently shown to improve bacterial elimination after preparation, but efforts have been expended towards developing intravisit antimicrobial supplementary approaches that could obviate the need for an interappointment medicament.

Optimized single-visit disinfection (OSD) approaches that supplement the effects of chemomechanical procedures include final canal rinse with CHX, mechanical, sonic, or ultrasonic activation of NaOCl, photodynamic therapy (PDT), and photon-induced photoacoustic streaming (PIPS) using Er:YAG laser energy at subablative power levels (Fotona, Ljubljana, Slovenia).

Mechanical, sonic, or ultrasonic activation of NaOCl following preparation has been recommended. Mechanical activation of NaOCl associated with a new supplementary instrument - XP-endo Finisher - has shown good results in terms of enhancing disinfection, but cannot predictably disinfect the isthmus area of molars. Sonic activation has shown no significant additional antimicrobial benefits. PUI is probably one of the most widely used supplementary approaches to optimize disinfection. Data from in vitro studies on PUI are rather inconclusive. Clinical trials reported no significant supplementary antibacterial effects of PUI. Moreover, an outcome study found no superior results for treatment using PUI. Clinical studies on the antibacterial benefits of a final rinse with CHX have also shown inconclusive results. So far, there is no consistent information from clinical studies about the antibacterial effectiveness of PDT and PIPS.

There are also OSD strategies that serve as an alternative to conventional chemomechanical procedures and include instruments especially designed to adjust to the root canal anatomy, the EndoVac system for negative pressure irrigation, and the GentleWave multisonic wave system (Sonendo, Laguna Hills, CA, USA).

The new instruments devised to deal with canals of irregular shapes include the Self-Adjusting File (SAF) system (ReDentNOVA, Ra’anana, Israel), TRUShaper (Dentsply Sirona, Tulsa, OK, USA), and XP-endo Shaper (FKG, La Chaux-de-Fonds, Switzerland). The latter two are very recent and there are no studies so far that have consistently evaluated their disinfecting ability in anatomically complex canals. In terms of cleaning, a study showed no significant difference between SAF, TRUShaper, and XP-endo Shaper in oval canals. The SAF system, in turn, has been extensively studied and seems to promote better cleaning, shaping, and disinfection of oval/flattened canals than do conventional rotary instruments. SAF has been shown to leave fewer untouched areas in oval canals: from 6% to 35%. Nevertheless, the SAF instrument performance is similar to that of conventional rotary NiTi instruments in round, narrow, and non-complicated canals.

The EndoVac system was shown by a clinical study not to significantly improve disinfection in comparison with conventional needle irrigation. As for the GentleWave system, there is no clinical study evaluating its antibacterial effectiveness.
Placement of a well-adapted filling material along the entire extent of the prepared canal may also help deal with the issue of unprepared surface areas. Entombed by the filling material, bacteria on unprepared surfaces may succumb. Actually, because bacterial persistence in the canal is a risk factor for poor treatment outcome, entombment is not reliable.132,133,134,135 In addition, bacteria in canal walls in contact with filling materials have been observed in some teeth with post-treatment apical periodontitis.97 Endodontic sealers usually exhibit some discrete antibacterial effects, but only before setting. Therefore, they should not be expected to significantly improve disinfection. In addition, because virtually no endodontic filling materials can promote a predictable antibacterial seal of the root canal,110,111,112,113 there will be instances when tissue fluids may leak into the canal and provide residual bacteria with nutrients. It is unclear whether leakage happens because not all walls were covered by sealer during obturation procedures or the sealer solubilized over time, resulting in a space fluids can leak into.104 Improvements in endodontic filling materials with better physicochemical features and antibacterial activity should be encouraged.

Conclusions

There is no consistent clinical evidence showing that OSD approaches can predictably reduce the bacterial bioburden to levels significantly below those achieved by conventional chemomechanical procedures. Some approaches still need to be tested. In addition to randomized clinical trials for evaluating the performance of many available systems and protocols, it is also important to use a correlation of analytical methods in *ex vivo* studies to better evaluate the cleaning and disinfecting effects of endodontic procedures in unprepared canal walls and difficult-to-reach areas. The development of systems, techniques, and strategies to improve cleaning and disinfection of untouched canal walls and difficult-to-reach areas should be encouraged with the purpose of improving the treatment outcome.

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Unprepared root canal surface areas: causes, clinical implications, and therapeutic strategies