Antimicrobial Therapeutics in Regenerative Endodontics: A Scoping Review

ABSTRACT

Introduction: This review aimed to provide a critical appraisal of alternative antimicrobial strategies in lieu of traditional triple antibiotic paste (TAP). Methods: This review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. The literature search was performed in 8 databases (PubMed/Medline, Embase, LILACS, Web of Science, Scopus, BVS, SciELO, and the Cochrane Library), selecting clinical, in vitro, in vivo, and in situ studies that evaluated antimicrobial alternatives to TAP in regenerative endodontics. Studies lacking an experimental TAP group were excluded. Results: A total of 1705 potentially relevant records were initially identified. From the 38 studies retrieved for full-text reading, 16 fulfilled all selection criteria and were included in the qualitative analysis. According to the study design, 11 studies were solely in vitro, 1 study was both in vitro and in vivo (animal model), 2 studies were solely animal experiments, and 2 studies were clinical trials. The alternative antimicrobial agents to TAP consisted of modified TAP formulations (eg, a combination of TAP with chitosan); TAP-eluting nanofibers; propolis; chlorhexidine (CHX) gels/solutions; double antibiotic pastes composed of distinct combinations of antibiotics; Ca(OH)₂-based formulations; and sodium hypochlorite. Overall, most of the alternative agents performed similarly to TAP, although some strategies (eg, Ca(OH)₂- and CHX-based formulations) seemed to present dubious importance in the control of infection. Conclusions: TAP still remains an excellent option in terms of the complete elimination of microorganisms. This review points to the use of electrospun fibers as a drug delivery system to offer a controlled release of the antimicrobial agent, as well as the use of natural compounds, deserving future investigation. (J Endod 2020;46:S115–S127.)

KEY WORDS

Antimicrobial agents; natural compounds; regenerative endodontics; scoping review

Regeneration of pulp tissue holds the promise of prolonged use of the natural dentition through predictable maintenance of dental root structure. Although the field of endodontics has been aware that healthy pulp tissue exists within infected/inflamed pulp, resective therapy has been the standard of care¹. However, in cases where pulp necrosis occurs in permanent teeth with open apex, the most commonly used treatment is apexification². This technique is based on the use of calcium hydroxide [Ca(OH)₂] or mineral trioxide aggregate (MTA) to induce hard tissue apical barrier formation³ and presents success rates that vary from 26% to 100%. Nonetheless, the major intrinsic disadvantage of this therapy is that these teeth have thin dentin walls, open apex, and, consequently, an increased risk of cervical fracture⁴,⁵. From a biological viewpoint, Ca(OH)₂ was demonstrated to induce cell proliferation as well as stem cell survival, although only under the use of low concentrations of that compound⁶,⁷. According to Khoshkhounnejad et al⁸, cell viability was significantly reduced on the basis of use of a Ca(OH)₂ paste (ie, the conventional form of the application of Ca(OH)₂ compounds as an intracanal dressing), which was explained by the notable cytotoxicity of a highly concentrated pasty consistency of Ca(OH)₂. It is noteworthy that Ca(OH)₂ and MTA-based materials may act as important antimicrobial agents in endodontics, although their bactericidal effects rely on the use of high concentration levels that could unavoidably impair cell survival⁹,¹⁰. Within this scenario, the field of regenerative endodontics has emerged and now provides a unique opportunity for exploring dentin-pulp complex regeneration. Thus, a more biological alternative to the apexification technique would help to prolong the maintenance of dental root structure by means of pulp

SIGNIFICANCE

There are several antimicrobial agents that share satisfactory antimicrobial activity with TAP, although there is still little evidence as to the best alternative strategy with considerably better disinfection ability than TAP.
alternatives to TAP, including but not limited to antibiotic-releasing nanofibers\textsuperscript{17–19,22,23}, distinct antibiotic formulations\textsuperscript{24–26}, and delivery vehicles\textsuperscript{27,28}. Last, although not recommended by the AAE, the combination of Ca(OH)\textsubscript{2} with chlorhexidine (CHX) has also been reported in clinical studies as an alternative to providing a better antimicrobial effect to Ca(OH)\textsubscript{2}\textsuperscript{29–32}. To provide a comprehensive update on progress in the field, this scoping review aimed to analyze antimicrobial strategies as an alternative to TAP for regenerative endodontics.

MATERIALS AND METHODS

This scoping review was reported, following as close as possible the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement\textsuperscript{33}, and the review focused on the following evidence-based practice question: What actual antimicrobial strategies could be an alternative to TAP in regenerative endodontics?

Search Strategies

Two independent reviewers conducted this literature search on studies published through July 2019. Eight databases were screened, including PubMed (Medline), Embase, LILACS, Web of Science, Scopus, BV5, SciELO, and the Cochrane Library. A search strategy was developed through MeSH terms, which were provided by Embase (Table 1) and adapted for other databases. The references cited in the included articles were also checked to identify other potentially relevant articles. Once articles from the databases were identified, they were imported into reference manager software (Mendeley Desktop, version 1.17.11; Mendeley Ltd, George Mason University, Fairfax, VA) to remove duplicates.

<table>
<thead>
<tr>
<th>Search terms</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1 (Infection Control) OR (Sterilization) OR (Disinfection) OR (Dental Sterilization) OR (Dental Disinfection) OR (Infection Control, Dental) OR (Dental Infection Control) OR (Control, Dental Infection) OR (Dental Infection Controls) OR (Infection Controls, Dental) OR (Anti-Infective Agents) OR (Agents, Anti-Infective) OR (Anti Infective Agents) OR (Anti-infective Agents) OR (Agents, anti-infective) OR (Microbicides) OR (Antimicrobial Agents) OR (Agents, Antimicrobial) OR (Antimicrobial Agents) OR (Agents, Anti-Microbial) OR (Anti-Microbial Agents) OR (Agents, Anti-Bacterial) OR (Anti-Bacterial Agents) OR (Antibacterial Agents) OR (Antibacterial) OR (Bacterial) OR (Bacterial Adhesion) OR (Dental Deposits) OR (Adhesion, Bacterial) OR (Antibacterial activity)</td>
</tr>
<tr>
<td>#2 (regenerative endodontics) OR (regenerative endodontic treatment) OR (regenerative endodontic procedure) OR (revitalization) OR (dental revascularization) OR (endodontic revitalization) OR (Root Canal Revascularization)</td>
</tr>
</tbody>
</table>

Eligibility Criteria

Two authors independently assessed all documents’ titles and abstracts. The inclusion criteria addressed clinical, in vitro, in vivo, and in situ studies that evaluated antimicrobial alternatives to TAP in regenerative endodontics. Not included in this review were studies that lacked a group consisting of a traditional TAP formulation to serve as a comparison. Also, the study should have used at least one antimicrobial analysis or an indirect evaluation capable of suggesting antimicrobial ability to determine whether one agent/compound would show better effectiveness than the other. Last, review articles, editorial letters, case reports, case series, and articles published in languages other than English, Portuguese, or Spanish were all excluded.

Study Selection

Full copies of all potentially relevant studies, including those appearing to meet inclusion criteria and those with insufficient data in the title and abstract to make a clear decision, were selected for full scrutiny. Two reviewers independently assessed in duplicate the full-text papers. Any disagreement regarding the eligibility of the included studies was resolved through discussion and consensus or by a third reviewer. Only articles that fulfilled all of the eligibility criteria were included.

Data Extraction and Analysis

The data were extracted by using a standardized form. If some information was missing, the authors were contacted via e-mail to retrieve it. The studies were categorized according to their design into the in vitro, animal model, and clinical trial categories. The following data were tabulated to the qualitative analysis of the in vitro studies: author, antimicrobial alternative agents used, the concentration of the antimicrobials, number of...
specimens investigated, antimicrobial test, type of specimens used, type of microorganisms, and the main findings of the study. Regarding animal experiments, information on the author, antimicrobial alternative agents used, the concentration of the antimicrobials, number of specimens, the analysis performed, type of tooth used, type of microorganisms evaluated, and the main findings of the study were all tabulated. Also, the following data from clinical trials were retrieved: author information, antimicrobial alternatives to TAP, number of subjects investigated, analyses performed, type of tooth used, and the main findings.

**RESULTS**

**Study Selection**

A total of 1704 potentially relevant records were identified from all databases, and 1 additional article was found by hand-search (Fig. 1). After removing duplicates, 1558 studies were screened by 2 reviewers with title/abstract evaluation. A total of 38 studies had the full-text assessed, with 22 being excluded because of the lack of adherence within the inclusion criteria: 14 studies were excluded because of the absence of a group consisting of a traditional TAP formulation for comparison purposes, and 8 studies were excluded because they did not investigate a new antimicrobial strategy feasible for regenerative endodontics application. Sixteen studies fulfilled all of the selection criteria and were included in the qualitative analysis. Tables 2–4 summarize the main information of studies categorized as in vitro, animal model, and clinical trials, respectively. According to the study design, 12 studies were in vitro, 3 studies involved an in vivo animal experiment, and 2 studies were clinical trials. All included studies were published between 2013 and 2019.

### In Vitro Studies

For the in vitro category, 12 studies were analyzed (Table 2). The alternative antimicrobial agents to traditional TAP consisted of modified TAP formulations (eg, a combination of TAP with chitosan), TAP-eluting nanofibers, propolis, CHX gels/solutions, double antibiotic pastes (DAP) composed of distinct combinations of antibiotics, calcium hydroxide-based formulations, and sodium hypochlorite (NaOCl). Compared with TAP, most of the foregoing alternative agents showed a similar or lower ability to inhibit bacterial/fungal growth. For instance, chitosan-modified TAP performed similarly to the TAP solution, although the concentration of formulations used was not revealed in the investigation. Studies that evaluated the inhibitory effects of DAP formulations demonstrated similar results to TAP, regardless of the combination of antibiotics used (ie, pastes that combined metronidazole [MET] with ciprofloxacin [CIP] or amoxicillin [AMOX]).

Propolis and NaOCl resulted in lower antimicrobial effects when compared with TAP (0.5 mg/mL), at least considering the tested concentrations. The TAP-eluting nanofibers...
### TABLE 2 - *In Vitro* Studies Selected in the Review Showing Antimicrobial Alternatives to TAP Use and Information on Concentration of Antimicrobials, Number of Specimens, Antimicrobial Test, Type of Specimens, Microorganisms, and Main Results of Each Study

<table>
<thead>
<tr>
<th>Study</th>
<th>Antimicrobial alternatives to TAP</th>
<th>Concentration of antimicrobials</th>
<th>n</th>
<th>Antimicrobial test/ type of specimens</th>
<th>Microorganism</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adl 2012²⁴</td>
<td>MET solution</td>
<td>25, 50, 100, and 200 μg/mL</td>
<td>6</td>
<td>Agar well diffusion assay/ BHI agar plates</td>
<td><em>E. faecalis</em></td>
<td>While the MET solution showed apparent similar inhibitory effect to bacterial growth as compared with TAP solution, regardless of the concentration, the CIP- and MINO-based solutions resulted in apparent lower inhibitory effect than TAP at each concentration level tested. Ca(OH)₂ solutions demonstrated lower inhibitory effect than TAP, and only on the 100 and 200 μg/mL concentration levels.</td>
</tr>
<tr>
<td>Albuquerque 2015⁵⁰</td>
<td>TAP-eluting nanofibers</td>
<td>25 mg/mL MET, CIP, and MINO</td>
<td>4</td>
<td>CLSM/biofilm-infected dentin</td>
<td><em>A. naeslundii</em></td>
<td>TAP-eluting nanofibers demonstrated slightly lower ability to kill bacteria (97.5% of dead bacteria) as compared with TAP solution (50 mg/mL), which killed 100% bacteria.</td>
</tr>
<tr>
<td>Albuquerque 2016⁵²</td>
<td>TAP-eluting nanofibers</td>
<td>25 mg/mL MET, CIP, and MINO</td>
<td>10</td>
<td>CFU/biofilm-infected dentin</td>
<td><em>P. gingivalis</em></td>
<td>TAP-eluting nanofibers and TAP solution (50 mg/mL) showed complete and similar ability to eliminate bacteria.</td>
</tr>
<tr>
<td>Albuquerque 2017⁵⁷</td>
<td>3D construct of TAP-eluting fibers</td>
<td>35 mg/mL MET, CIP, and MINO</td>
<td>4</td>
<td>CLSM/biofilm-infected dentin</td>
<td><em>A. naeslundii</em></td>
<td>3D TAP-eluting construct exhibited similar ability to kill bacteria as compared with TAP solution (50 mg/mL).</td>
</tr>
<tr>
<td>Bottino 2019³⁸</td>
<td>3D construct of TAP-eluting fibers</td>
<td>35 mg/mL MET, CIP, and MINO</td>
<td>4</td>
<td>CLSM/biofilm-infected dentin</td>
<td><em>A. naeslundii</em></td>
<td>3D TAP-eluting construct exhibited similar ability to kill bacteria as compared with TAP solution (50 mg/mL).</td>
</tr>
<tr>
<td>Chua 2014³³</td>
<td>95% propolis (Stakich) 2% CHX gel (Consapex V) Ca(OH)₂ + propylene glycol paste</td>
<td>Not informed</td>
<td>5 CFU/infected dentin</td>
<td><em>C. albicans</em></td>
<td>Only the most concentrated TAP solution (10 mg/mL) reduced total amount of viable bacteria. All alternative antimicrobial agents resulted in reduced counts of viable bacteria, although without statistical significance, except for 10 mg/mL-based DAP solution, which eliminated almost 74% of bacteria.</td>
<td></td>
</tr>
<tr>
<td>Latham 2016²⁵</td>
<td>DAP Ca(OH)₂ (UltraCal XS; Ultradent)</td>
<td>10, 1, and 0.1 mg/mL MET and CIP</td>
<td>8</td>
<td>CFU/infected dentin</td>
<td><em>E. faecalis</em></td>
<td>All DAP solutions demonstrated similar inhibitory effects of bacteria as compared with TAP solution, except for DAP solution combined with Ca(OH)₂, which did not result in any inhibitory effects. Ca(OH)₂ solutions did not show inhibitory effect against bacteria.</td>
</tr>
<tr>
<td>Maniglia-Ferreira 2016²⁷</td>
<td>DAP I + Ca(OH)₂</td>
<td>175 mg/mL</td>
<td>3</td>
<td>Agar-disk diffusion assay/BHI agar plates</td>
<td><em>E. faecalis</em></td>
<td>The TAP used (~60 mg/mL) showed greatest reduction of viable bacteria (98.6% of dead bacteria), which was greater than that obtained from Ca(OH)₂ or CHX administration (96.3% and 71.7% of dead bacteria, respectively).</td>
</tr>
<tr>
<td>Ordinola-Zapata 2013²⁸</td>
<td>TAP Ca(OH)₂ 2% CHX gel</td>
<td>500 mg of each antibiotic</td>
<td>10</td>
<td>CLSM/intraorally biofilm-infected dentin</td>
<td>Oral pathogens from a healthy volunteer</td>
<td>All DAP solutions demonstrated similar inhibitory effects of bacteria as compared with TAP solution, except for DAP solution combined with Ca(OH)₂, which did not result in any inhibitory effects. Ca(OH)₂ solutions did not show inhibitory effect against bacteria.</td>
</tr>
<tr>
<td>Pankajakshan 2016²⁶</td>
<td>TAP-eluting nanofibers</td>
<td>30 mg/mL MET, CIP, and MINO</td>
<td>4</td>
<td>CLSM/biofilm-infected dentin</td>
<td><em>A. naeslundii</em></td>
<td>TAP-eluting nanofibers and TAP solution (50 mg/mL) presented similar ability to kill bacteria (95.5% vs 93.3% of dead bacteria, respectively).</td>
</tr>
<tr>
<td>Shaik 2014³⁵</td>
<td>TAP + chitosan Ca(OH)₂ + chitosan Ca(OH)₂ solution</td>
<td>Not informed</td>
<td>5 CFU/infected dentin</td>
<td><em>C. albicans</em></td>
<td>All alternative agents resulted in similar antifungal and antibacterial effects when compared with TAP solution.</td>
<td></td>
</tr>
</tbody>
</table>

(continued on next page)
demonstrated an overall similar ability to kill bacteria, as compared with traditional TAP and on the use of lower concentrations of the antibiotics (eg, 25–35 mg/mL vs 50 mg/mL of TAP solution). No less important, Ca(OH)$_2$-based compounds performed distinctly in relation to TAP, showing a lesser ability to kill bacteria when low-concentrated formulations were considered (eg, 25–200 µg/mL), as well as negative antimicrobial potential against Enterococcus faecalis when administered using saline solution or a 2% CHX solution as the vehicle$^{27}$. However, some studies demonstrated that Ca(OH)$_2$-based formulations may perform similarly to TAP if combined with chitosan$^{35}$, propylene glycol and calcium carbonate$^{36}$, or the commercial ingredients of UltraCal XS (Ultradent, South Jordan, UT), ie, radiopacifiers, water, and methylcellulose$^{37}$. To test the inhibitory effects of the antimicrobial agents, most of the studies used biofilm-infected dentin specimens. Concerning the antimicrobial tests, 5 studies used confocal laser scanning microscopy to identify the percentage of live and dead bacteria, 5 studies used the colony-forming unit (CFU/mL) method for detecting the approximate counts of viable bacteria, and 3 studies used the agar diffusion method. Seven studies (~58%) used E. faecalis for the preparation of the infected specimens, followed by other microbial species such as Actinomyces naeslundii (~33%), Candida albicans (16.7%), Porphyromonas gingivalis (8.3%), and Streptococcus sanguinis (8.3%). Only one study$^{38}$ used an intraradical model (in situ) for the preparation of the infected specimens; therefore, the specimens were infected with an unknown variety of microbial. Most studies informed the concentration of antibiotics used for the preparation of the TAP solution, which ranged from 0.5 mg/mL to 60 mg/mL of each antibiotic.

### Studies That Used an In Vivo Animal Model

For the in vivo animal category, 3 studies were analyzed (Table 3). All the studies considered a beagle dog model and used immature permanent double-rooted teeth from both the upper and lower premolars that had necrotic pulps/apical periodontitis. Alternative antimicrobial strategies to TAP varied from a 3-dimensional (3D) tubular-shaped TAP-eluting nanoﬁbrinous construct containing 35 mg/mL of each antibiotic$^{18}$ to a 1% propolis paste$^{37}$ or Qmix solutions composed of CHX, EDTA, and a surfactant detergent$^{38}$. All the teeth were infected following a direct contamination process, ie, the teeth were incorporated with supragingival plaque scaled from the dogs’ teeth or they were left open for a certain number of days. Two studies$^{18,37}$ used a histopathologic analysis to examine the healing outcomes (ie, apical root closure, thickness of the dentin layer, and presence or absence of inflammatory reaction around the apical zone), whereas one study$^{38}$ used the CFU analysis to verify the antimicrobial potential of the alternative strategies to reduce bacterial counts as compared with TAP. Overall, the use of both the 3D TAP-eluting construct and propolis paste resulted in similar tooth maturation events when compared with TAP administration and also lower toxicity or potential to provoke inflammatory reactions in the periapical area than TAP. Considering the antimicrobial effects of the strategies, TAP demonstrated a similar ablation of intracanal biofilm as compared with the 3D TAP-eluting construct, but significantly greater potential to reduce bacterial counts when compared with the unmodified Qmix solution or the CHX-modified Qmix solution that was tested. Last, all studies used NaOCl as an irrigant solution, with concentrations ranging from 1% to 5.25%.

### Clinical Studies

This review analyzed 2 in vivo clinical trials (Table 4). The alternative antimicrobial strategy was the same for both studies (ie, a mixture composed of Ca(OH)$_2$ paste and a 2% CHX formulation), differing only in the CHX vehicle used, solution$^{27}$ or gel$^{36}$. The study by Arruda et al$^{29}$ evaluated 48 subjects, whereas in the study by Nagata et al$^{38}$, only 23 subjects were investigated. Whereas the former tested the antimicrobial agents in mature permanent teeth, the latter performed the clinical trial using immature permanent teeth. One study$^{30}$ performed a quantitative polymerase chain reaction (qPCR) analysis so that the counts of bacteria were assessed before and after the application of the regenerative protocols. TAP reduced the bacterial counts by 97%, which was significantly greater than that obtained with the application of the Ca(OH)$_2$/CHX paste (39%), and the qPCR negative samples were more frequently observed in the TAP group. The other study$^{31}$ performed clinical and radiographic examinations on the treated teeth and showed that both TAP and Ca(OH)$_2$/CHX paste were effective in reducing pain-related conditions, although the former led to a greater occurrence of crown discoloration (83.3% vs 27.3%, respectively).

### DISCUSSION

Currently, there are 3 antimicrobial formulations recommended by the AAE for the disinfection of immature permanent teeth with necrotic pulps:
<table>
<thead>
<tr>
<th>Study</th>
<th>Antimicrobial alternatives to TAP</th>
<th>Concentration of antimicrobials</th>
<th>n</th>
<th>Analysis/type of tooth</th>
<th>Type of microorganisms</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottino 2019</td>
<td>3D TAP-eluting construct</td>
<td>35 mg/mL MET, CIP, and MINO</td>
<td>4</td>
<td>Histopathologic examination/immature permanent double-rooted upper and lower premolars with necrotic pulps</td>
<td>Biofilm consisting of supragingival plaque scaled from dogs’ teeth</td>
<td>3D TAP-eluting construct was effective in ablatting intracanal biofilm in similar fashion to TAP, allowing apical root closure and ingrowth of thin layer of osteodentin-like tissue into the root canal. Also, the alternative strategy seemed to provoke less intense inflammatory reaction as compared with TAP.</td>
</tr>
<tr>
<td>Pagliarin 2016</td>
<td>TAP 1% propolis paste</td>
<td>20 mg/mL MET, CIP, and MINO</td>
<td>10</td>
<td>Histopathologic examination/immature permanent double-rooted upper and lower premolars with necrotic pulps</td>
<td>Microorganisms derived from direct contamination (ie, teeth were left open for 3 weeks)</td>
<td>There were no significant differences between the alternative antimicrobial strategy and TAP regarding new mineralized tissue deposition, although use of propolis paste increased tissue deposition on dentinal walls. Tissue vitality was greater inside the root canals on the use of propolis paste, probably because of its minimum toxicity. Conversely, TAP content used in this study (20 mg/mL of each antibiotic) led to severe inflammatory reactions in 50% of samples. Association between 5.25% NaOCl irrigation using Endovac system and final irrigation with Qmix solution may result in higher bacterial reduction than 2 treatment sessions using intracanal dressing of 2% CHX gel or TAP. However, if intracanal dressing must be used, application of TAP (20 mg/mL of each antibiotic) is more effective in bacterial reduction than CHX dressing.</td>
</tr>
<tr>
<td>Rodríguez-Benítez 2014</td>
<td>TAP Qmix solution*</td>
<td>20 mg/mL MET, CIP, and MINO</td>
<td>10</td>
<td>CFU/immature permanent double-rooted upper and lower premolars with apical periodontitis</td>
<td>Microorganisms derived from direct contamination (ie, teeth were left open for 7 days and then kept sealed for 15–25 days with a coronal sealer)</td>
<td></td>
</tr>
</tbody>
</table>

Qmix, mixture containing CHX, EDTA, and a surfactant detergent.

*Root canals were flushed with 1 mL of the solution for 90 seconds.
†The gel was placed as intracanal dressing for 15 days.
Table 4: Studies Selected in the Review That Used Clinical Trial Model Showing Antimicrobial Alternatives to TAP Use and Information on Concentration of Antimicrobials, Number of Subjects Investigated, Analyses Performed, Type of Tooth, Microorganisms, and Main Results of Each Study

<table>
<thead>
<tr>
<th>Study</th>
<th>Type of tooth</th>
<th>Antimicrobials to TAP</th>
<th>Analysis performed</th>
<th>n</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arruda 2018</td>
<td>Immature permanent single-rooted teeth with single canal presenting carious lesion and necrotic pulp</td>
<td>Ca(OH)₂ paste + 2% CHX solution</td>
<td>qPCR assay</td>
<td>48</td>
<td>Bacterial counts were reduced by 97% after application of TAP. Ca(OH)₂ paste was more effective in reducing bacterial counts when compared with CHX-gel. Both revascularization therapies were effective in reducing spontaneous pain, percussion and palpation sensitivity.</td>
</tr>
<tr>
<td>Albuquerque et al</td>
<td>Immature permanent single-rooted upper teeth with necrotic pulp</td>
<td>Ca(OH)₂ paste + 2%</td>
<td>Clinical and radiographic findings</td>
<td>23</td>
<td>Significantly lower (39%). Quantitative PCR negative samples were more frequently observed in TAP group. Both revascularization therapies were effective in reducing spontaneous pain, percussion and palpation sensitivity. Ca(OH)₂ paste led to greater occurrence (83.3%) of crown discoloration than the CHX-gel (35.4%).</td>
</tr>
</tbody>
</table>

**New Alternatives in the Concept**

**Antibiotic-Eluting Fibers as Drug Delivery Systems**

The undesired cytotoxic effects of TAP on dental pulp stem cells have been reported as one of the major drawbacks of the revascularization protocol17,19,38,40, and with the greater concentration of each antibiotic added to TAP, the lower its cytocompatibility. Nevertheless, the outstanding antimicrobial potential of TAP is an important feature that offers satisfactory elimination of infection, which is essential to allow proper healing and complete maturation of the teeth, so that any other alternative intracanal medication should demonstrate a similar or better ability to combat infection as compared with TAP, and hopefully with enhanced biocompatibility. Remarkably, the incorporation of TAP ingredients into small-sized polymeric and biodegradable fibers processed via electrospinning has been proposed as a more cell-friendly approach because of the considerably lower content of antibiotics found within the fibers31,32,39,41–44. Indeed, the TAP-eluting nanofibers are able to release the antibiotics following a well-controlled scenario, with confirmed antimicrobial properties, as demonstrated by the studies included in this review. On the basis of 5 studies that reported on the use of TAP-eluting nanofibers for the disinfection of biofilm-infected dentin samples, 4 showed similar antimicrobial activity as compared with TAP17,18,32,34, the other study32 exhibited a slightly lower ability to kill bacteria (97.5%) compared with TAP (100%), although it may still be considered an excellent result. One should consider the concentration of antibiotics used to fabricate the TAP-eluting nanofibers in those studies, which ranged from 25 to 35 mg/mL, as being lower than the amount used to prepare the paste-like TAP (50 mg/mL). Also, considering that thousands of fibers may be obtained in the electrospun fibrous constructs, this may suggest that the final amount of drugs existing within each fiber is minimal but still effective in the elimination of infection32,39,41. In fact, in the study by Albuquerque et al., tooth discoloration was lower on the use of the TAP-eluting nanofibers when compared with the use of TAP, once again suggesting the lower content of antibiotics within the former. The foregoing aspects can be better observed in Figure 2A, which shows the drug release profile of TAP antibiotics from the TAP-eluting nanofibers fabricated in the study by Albuquerque et al.32 after 28 days of incubation. Despite the occurrence of a burst release of drugs during the first days of incubation, the percentage of drugs released was not complete, thus suggesting the existence of some amount of antibiotic within the fibers (ie, probably because of a crosslinking effect with the polymeric structure of the fibers) that could be available for long-term release. In this respect, one may consider that the lower concentration of drugs found in the TAP-eluting nanofibers, as well as their slower release rate, may contribute to the lower staining effect on dentin. 

Ca(OH)₂, traditional TAP, and modified TAP formulations, with the latter comprising a mixture of 3 antibiotics lacking in MINO, which should be replaced by an alternative antibiotic to prevent tooth discoloration, or a mixture containing only 2 antibiotics different from MINO (DAP). Here, it is worth mentioning that all the foregoing disinfectants possess important drawbacks that may hamper the revascularization process. For instance, Ca(OH)₂ may present limited antimicrobial activity against some types of microorganisms, especially E. faecalis and C. albicans23,32; this may provide neither antimicrobial properties nor bactericidal activity under low concentration levels9, and it does not induce tooth maturation, thus reducing tooth strength and increasing the occurrence of root fractures over time. Regarding the use of antibiotic pastes, cytotoxicity is a major concern because antibiotics may interfere with stem cell viability, thus impairing the regenerative process itself; no less important is that antibiotics may create bacterial resistance in the microorganisms, therefore diminishing their therapeutic effects. Last, some antibiotics (eg, MINO) may provoke intense crown discoloration, thus impairing the regenerative process itself; no less important is that antibiotics may create bacterial resistance in the microorganisms, therefore diminishing their therapeutic effects. 

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New Alternatives in the Concept

Antibiotic-Eluting Fibers as Drug Delivery Systems

The undesired cytotoxic effects of TAP on dental pulp stem cells have been reported as one of the major drawbacks of the revascularization protocol17,19,38,40, and with the greater concentration of each antibiotic added to TAP, the lower its cytocompatibility. Nevertheless, the outstanding antimicrobial potential of TAP is an important feature that offers satisfactory elimination of infection, which is essential to allow proper healing and complete maturation of the teeth, so that any other alternative intracanal medication should demonstrate a similar or better ability to combat infection as compared with TAP, and hopefully with enhanced biocompatibility. Remarkably, the incorporation of TAP ingredients into small-sized polymeric and biodegradable fibers processed via electrospinning has been proposed as a more cell-friendly approach because of the considerably lower content of antibiotics found within the fibers31,32,39,41–44. Indeed, the TAP-eluting nanofibers are able to release the antibiotics following a well-controlled scenario, with confirmed antimicrobial properties, as demonstrated by the studies included in this review. On the basis of 5 studies that reported on the use of TAP-eluting nanofibers for the disinfection of biofilm-infected dentin samples, 4 showed similar antimicrobial activity as compared with TAP17,18,32,34, the other study32 exhibited a slightly lower ability to kill bacteria (97.5%) compared with TAP (100%), although it may still be considered an excellent result. One should consider the concentration of antibiotics used to fabricate the TAP-eluting nanofibers in those studies, which ranged from 25 to 35 mg/mL, as being lower than the amount used to prepare the paste-like TAP (50 mg/mL). Also, considering that thousands of fibers may be obtained in the electrospun fibrous constructs, this may suggest that the final amount of drugs existing within each fiber is minimal but still effective in the elimination of infection32,39,41. In fact, in the study by Albuquerque et al., tooth discoloration was lower on the use of the TAP-eluting nanofibers when compared with the use of TAP, once again suggesting the lower content of antibiotics within the former. The foregoing aspects can be better observed in Figure 2A, which shows the drug release profile of TAP antibiotics from the TAP-eluting nanofibers fabricated in the study by Albuquerque et al.32 after 28 days of incubation. Despite the occurrence of a burst release of drugs during the first days of incubation, the percentage of drugs released was not complete, thus suggesting the existence of some amount of antibiotic within the fibers (ie, probably because of a crosslinking effect with the polymeric structure of the fibers) that could be available for long-term release. In this respect, one may consider that the lower concentration of drugs found in the TAP-eluting nanofibers, as well as their slower release rate, may contribute to the lower staining effect on dentin.
as compared with the more concentrated conventional TAP observed in Figure 2B. Moreover, cytotoxicity could be considerably lower under application of the TAP-eluting nanofibers, because the total amount of drugs released is lower than TAP.

Other studies retrieved for full-text reading but that failed to meet the eligibility criteria because of their lack of using an experimental group composed of TAP also demonstrated that electrospun fibers loaded with CIP and/or MET showed significant inhibition of biofilm growth. In one study, CIP and MET were incorporated at the 5 or 25 mg/mL concentration level, whereas the CIP-loaded fibers demonstrated antibacterial activity against both E. faecalis and P. gingivalis, regardless of the concentration, as expected the MET-loaded fibers were only effective against the latter type of microorganism. This may reinforce the use of more than 1 type of antibiotic to increase the antimicrobial activity of the intracanal dressing, thus explaining the good acceptability of the DAP and TAP formulations. Another study, which prepared bimix CIP/MET-releasing nanofibers with varying amounts of each antibiotic, showed antimicrobial efficacy against E. faecalis, P. gingivalis, and Fusobacterium nucleatum and reduced impact on cell viability.

Overall, it seems that electrospun nanofibers may act as optimal carriers/vehicles for the slow release of antibiotics, showing potential application for the disinfection of contaminated root canals. It is worth mentioning that 3D tubular-shaped TAP-eluting nanofibrous constructs have recently demonstrated by using an in vivo dog model to properly fit within the volume of the root canal, resulting in satisfactory infection control and healing of the periapical tissues, thus inducing apex closure. This may be an important step for the translation of this outstanding approach to the clinic, which may truly become a viable antimicrobial strategy to traditional TAP.

**Natural Compounds**

There is a great variety of natural compounds that could be interestingly applied in root canal disinfection. Propolis is one example because of its good antimicrobial and anti-inflammatory properties associated with it containing flavonoids, as well as phenolic, aromatic, and diterpene acids. Propolis is a sticky resinous compound that honeybees collect from buds and the exudates of plants. Also, their antifungal and antimicrobial activity is well-known in the literature. One of the included studies evaluated propolis as an alternative to the actual medicaments used in regenerative endodontics. The antifungal effects of the medicaments were tested against C. albicans at 2 time points, ie, after 1 and 7 days of application of the materials as an intracanal dressing. Although after 24 hours propolis was less effective than TAP and the other medicaments tested (2% CHX-based gel and Ca(OH)2 paste mixed with propylene glycol), after 7 days the medications were all equally effective compared with TAP.

According to the present review, one in vivo study using a dog model tested the effects of a 1% propolis paste as an intracanal dressing in the revascularization technique. The propolis paste was compared with traditional TAP, and the authors demonstrated that both protocols allowed similar deposition of new mineralized tissue. However, while the TAP-treated teeth resulted in considerably greater events of severe inflammatory reactions, the propolis-treated teeth revealed the minimal presence of inflammatory cells and an increased tissue deposition on dentin walls, thus indicating minimum toxicity behavior of the latter compared with TAP. This finding was also corroborated by another study by Zarei et al., in which the application of propolis as an intracanal dressing in a dog model induced expression of vascular endothelial growth factor and VIII factor in infected mature and immature teeth, suggesting a bioactive property for propolis in the healing of nonvital teeth.

**Well-Established Antimicrobial Options**

**Antibiotic Mixtures**. Antibiotic pastes consisting of the combination of 2 or 3 antibiotics free of MINO have been proposed.
as adequate intracanal dressings for the disinfection of necrotic immature teeth and show much better color stability for treated teeth after application of the material. In this review, some of the studies that were included tested the antimicrobial activity of different DAP/TAP formulations. For example, in the studies by Latham et al.25 and Valverde et al.36, DAP was prepared by mixing MET and CIP antibiotics, ie, the most commonly used DAP composition. Conversely, the study by Maniglia-Ferreira et al.37 tested the antimicrobial effects of 2 distinct DAP formulations, namely the conventionally used and one modified version (DAP I) in which CIP was replaced by amoxicillin (AMOX). In the latter study, the DAP pastes were kept unmodified or they were also incorporated with other ingredients such as Ca(OH)2 or ZnO.

In the study by Latham et al.25, DAP was compared with TAP at 3 different concentration levels (10, 1, and 0.1 mg/mL). Only the most concentrated antibiotic mixtures significantly reduced E. faecalis counts, although the reduction obtained with TAP administration was greater (100%) than that obtained with DAP (74%). This finding may elucidate 2 important aspects: concentration levels ≤ 1 mg/mL seem to be ineffective in eliminating this type of bacteria, which is one of the most prevalent microorganisms found in necrotic pulps, and the antibacterial effect of DAP is reduced when compared with TAP, thus suggesting that the presence of MINO or another antibiotic with similar spectra action is necessary to improve the disinfection potential of the intracanal medication. Remarkably, when DAP was prepared by mixing MET and AMOX (DAP I), the antimicrobial effect against E. faecalis was statistically similar to that from TAP27, but different from the results found for the conventional DAP formulation, which resulted in lower inhibition halos as compared with TAP, although they could still be considered adequate. It seems that AMOX possesses greater antimicrobial activity than CIP for the elimination of root canal infections, which may be related to their main mechanisms of action, ie, whereas AMOX acts at the cellular level of bacteria, CIP acts at the intracellular level by inhibiting the synthesis of DNA, so that the former has possibly an enhanced and faster-killing action than the latter.

One important aspect of the study by Maniglia-Ferreira et al.37 is that the authors have also modified the DAP and DAP I antibiotic mixtures with the incorporation of Ca(OH)2 or ZnO particles. Surprisingly, the presence of Ca(OH)2 in the DAP formulation resulted in excellent inhibition halos, which were similar to those obtained with unmodified DAP and TAP medications. On the other hand, when Ca(OH)2 was mixed with the DAP I formulation, some negative chemical interactions must have occurred between AMOX and the particles because no inhibition halos were observed on administration of this mixture. The combination of ZnO with conventional DAP resulted in inhibition halos that were lower than those from unmodified DAP or TAP, although without statistical significance. Thus, the authors suggested that the incorporation of inorganic particles such as Ca(OH)2 or ZnO is not necessary to enhance the antimicrobial effects of the antibiotic pastes. However, whereas the DAP mixture tended to produce yellow coloring on samples, the presence of the particles induced dark coloring in the Ca(OH)2-treated samples and a colored-stable appearance in the ZnO-treated samples. According to the authors, this aspect must be considered when esthetics are paramount.

No less important is the study by Valverde et al.36, which also compared the antimicrobial effects of DAP with TAP, although both medications showed a similar reduction in bacterial growth. Here, one may consider that if the concentration of the antibiotic mixtures was low, ie, 0.5 mg/mL, which is below the concentration indicated by the AAE (1 mg/mL), new studies should focus on the investigation of low-concentration antibiotic mixtures to elucidate whether there is a minimum concentration threshold for the proper antimicrobial effect.

Ca(OH)2-Based Compounds. Although some studies reveal that Ca(OH)2 may present effective antimicrobial activity against a variety of microorganisms, probably because of their alkaline characteristics, other studies have shown that these compounds lack in the disinfection ability of contaminated root canals. Indeed, the study by Adl et al.24 and Maniglia-Ferreira et al.37, in which Ca(OH)2 was administered using saline or 2% CHX as dressing vehicles, demonstrated no antimicrobial effects against E. faecalis. Of note, the former study prepared low-concentration solutions, ranging from 25 to 200 μg/mL, and the latter considered Ca(OH)2-based solutions that were much more concentrated (175 mg/mL), and despite this difference in concentrations between the studies, both resulted in negative antimicrobial activity of the medicaments. Perhaps the antimicrobial test used in the foregoing studies (agar diffusion assay) was less sensitive than the methodology used in the study by Ordinola-Zápata et al.38, which used confocal laser scanning microscopy analysis in a biofilm-infected dentin model so that the Ca(OH)2 solution was able to eliminate bacteria, but still under considerably lower effectiveness compared with TAP (36.3% vs 98.6%, respectively). However, according to the authors, the alkaline effects of Ca(OH)2-based compounds are not likely effective in killing bacteria, especially in the form of biofilms, as probably explained by the overall amount of hydroxyl ions that are not suitably high enough to promote antimicrobial activity.

Different from the findings of the previous studies, the antimicrobial activity of the Ca(OH)2-based compounds seemed to improve when these particles were added in more complex formulations. For instance, the commercial UltraCal paste (Ultradent Products, Inc., South Jordan, UT) demonstrated a similar ability to kill bacteria as compared with TAP25, which may be a consequence of the heterogeneous composition of this paste, ie, a mixture of Ca(OH)2, ZnO, colophony, and polyethylene glycol. Also, when Ca(OH)2 was combined with chitosan, a natural product broadly used as a drug carrier49, the antifungal (C. albicans) and antimicrobial (E. faecalis) activity of this formulation was similar to that of TAP25. Last, the study by Valverde et al.36 demonstrated that a dental base paste composed of Ca(OH)2, propylene glycol, and calcium carbonate at the 2:5:3 proportion ratio led to a similar reduction in the counts of E. faecalis up to 7 days. Similarly, the incorporation of Ca(OH)2 into a propylene glycol paste led to similar antifungal activity to TAP against C. albicans33.

Overall, when Ca(OH)2 was combined with saline or CHX solutions, there was no antimicrobial action, but if the Ca(OH)2 particles were combined with other specific ingredients, their disinfection ability was synergistically enhanced. This should be investigated in future studies to verify the real impact that Ca(OH)2 particles, applied under distinct formulations and vehicles, may have on the healing process of infected immature teeth with necrotic pulp. It is also worth mentioning that according to the clinical studies included in this review, application of a Ca(OH)2-based paste combined with CHX may not present the same level of disinfection compared with TAP27, but it may still be as effective as TAP in terms of clinical and radiographic periapical healing50.

CHX-Based Formulations. Chlorhexidine is a commonly used dental antiseptic agent that has both bacteriostatic action at low-concentrations and a bactericidal action at high concentrations, also showing optimal efficacy due to its substantivity-antimicrobial effects may remain active up to 3 months50. According to the studies included in the present review, CHX was used at 2
concentration levels, a low-concentrated 0.2% CHX solution or a highly concentrated 2% CHX gel/solution. Despite CHX-based mixtures not being considered standard options as indicated by the AAE, it is possible to understand that most of the included studies designed at least 1 experimental group with CHX, suggesting the broad use of this agent for that purpose. It is noteworthy that the only 2 clinical studies that met the eligibility criteria in this review were aimed to compare the clinical effectiveness of TAP and a CHX-based formulation, which deserved discussion.

Concerning the use of 2% CHX gel, this review gathered the findings of 4 studies: 2 in vitro studies, 1 in vivo study using an animal model, and 1 clinical trial. The in vitro findings showed controversial results, in which the application of 2% CHX gel resulted in a significantly lower ability to kill bacteria compared with TAP (71.7% vs 98.6% respectively) or an apparently greater compared with TAP (71.7% vs 98.6%, significantly) or an apparently greater disinfection ability than TAP. Considering that the concentration of TAP was not revealed in both studies, their different findings cannot be further compared with each other. However, in the in vivo study, which may offer a more relevant scenario to test the effectiveness of different intracanal medicaments, one group, consisting of irrigation with a Qmix solution based on CHX followed by the application of a 2% CHX gel, resulted in greater counts of microorganisms as compared with TAP, indicating lower efficacy of the former when compared with the latter. Importantly, in the clinical study that compared the effects of a Ca(OH)₂ paste combined with 2% CHX gel, the use of this revascularization protocol led to acceptable healing of the periapical tissues, showing similar clinical and radiographic data compared with teeth treated with TAP. The repair obtained with both protocols indicated their effectiveness in controlling the infection, because repair may only occur under organized and uninfection tissue circumstances. Moreover, tooth discoloration was more pronounced after the use of TAP, which can be considered an advantage of the Ca(OH)₂/CHX paste when compared with TAP.

The findings obtained from the studies that used a 2% CHX solution as an antimicrobial agent showed a disappointing scenario because this approach was always associated with lower disinfection ability than TAP. Even in the clinical study by Arruda et al, the teeth treated with the Ca(OH)₂/CHX paste were disinfected by only 30%, differing from the TAP-treated teeth, which were almost completely decontaminated (97% of dead bacteria). One may consider that the use of 2% CHX solutions, combined or not with Ca(OH)₂ particles, is not the most advisable antimicrobial strategy for infection control in the case of necrotic immature teeth. Last, considering the findings from the study that used the low-concentration 0.2% CHX solution, it is possible to observe that the combination of this solution with an experimental dental base paste containing calcium resulted in the same antibacterial effects of TAP against E. faecalis biofilm, which is in accordance with the literature and corroborates to the well-recognized antimicrobial effects of CHX at this concentration level.

New Trends for Future Perspective

During the present study’s systematic review process, some studies retrieved for full-text reading that failed to fulfill the eligibility criteria have also revealed some interesting alternative antimicrobial agents that could potentially be used in lieu of TAP. Even though these studies did not use TAP as an experimental group, their information was gathered to present some future perspectives of novel formulations with potential applicability in regenerative endodontics.

Chitosan

Chitosan is a versatile natural compound that has been widely used as a drug carrier, showing good biocompatibility, degradability, and nontoxicity. According to the U.S. Food and Drug Administration, chitosan is an excellent wound dressing material. One study evaluated chitosan as a carrier of TAP and Ca(OH)₂ particles. Both combinations showed similar performance to each other, and when compared with comparable formulations in which the same medicaments were applied using saline as a vehicle, the chitosan-based compounds were significantly more effective than the latter. This finding was explained by the possible antimicrobial mechanism of action that is inherent in the chitosan molecule, wherein the positively charged NH₃⁺ groups of glucosamine found in its structure may interact with the surface components of negatively charged bacteria, leading to bacterial leakage and damage to vital activities.

Mesoporous Bioactive Glasses

Bioactive glasses have attracted significant attention because they can be functionalized with antimicrobial and mineralizing agents. Mesoporous bioactive glasses (MBGs) are a new class of material that has been developed; they are being used as drug delivery systems, especially for bone tissue regeneration. One study synthesized Ag-MBGs and evaluated the antibiofilm activity of this compound as an intracanal disinfectant. The release of Ag⁺₂ ions through functionalization gave Ag-MBGs an antibacterial effect. The good antibacterial action of Ag-MBGs against E. faecalis biofilm was attributed to the leaching of Ag⁺₂ ions.

Hydrogel Scaffolds

Three animal studies estimated the presence of bacteria inside the root canal via histologic analysis after the application of an intracanal medication, compared with the evoked bleeding approach associated with hydrogel scaffolds with the intention of promoting endodontic disinfection and investigating the capacity of tissue regeneration. In these studies, the presence of newly formed mineralized tissue was observed in the specimens with either small or no amounts of bacteria, with no differences noted between treatments. On the other hand, no pulp tissue formation was observed. In cases where the treatment failed and was even devoid of bacteria, the cytotoxic potential of the antimicrobials was linked with the possibility of residual bacterial infection, which could promote an inflammatory stimulus, affect properties of the remaining dentin, and consequently impact the tissue regeneration process. However, this approach deserves further investigation.

CONCLUSION

On the basis of the available literature, it is challenging to point to a protocol that has more predictable results in regenerative endodontics than traditional TAP. Despite all of the discussed limitations related to TAP use, it still remains an excellent option in terms of the complete elimination of microorganisms. Disinfection of necrotic immature teeth represents a challenge because of the complex anatomy of the root canals and the different susceptibility of microorganisms to antimicrobial agents used as disinfectants. This review points to the use of electrospun fibers as a drug delivery system to offer a controlled release of the antimicrobial agent. Even though only TAP ingredients were added to electrospin fibers, the possibility of incorporating natural compounds with a potent ability to combat endodontic infections is paramount and deserves future investigation, aiming for the major goal of offering a fully biocompatible
approach that eradicates infection and induces optimal tooth maturation and periapical tissue healing. Therefore, well-designed in vitro and in vivo studies, especially randomized clinical trials, are necessary to confirm the use of alternative antimicrobial agents in lieu of the traditionally used TAP, which may have an important impact on the quality of regenerative endodontics.

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REFERENCES


