Regenerative/revitalisation endodontic techniques are increasingly used as a treatment approach for the management of immature permanent teeth with necrotic pulps. Different chemical irrigants and medicaments are routinely used clinically for intra-canal disinfection. However, despite remarkable progress in this field, coronal discolouration, cell cytotoxicity, difficulty of removal of organic biofilm from the root canal, development of sensitisation and antimicrobial resistance are still challenges to this line of treatment. This review critically discusses and challenges the current status quo of antimicrobials used in regenerative endodontics and sheds the light on future alternative antimicrobial materials with regenerative potential.

Keywords: Antimicrobials, antibiotics, disinfection, biomaterials, regenerative endodontics, drug delivery, dental pulp stem cells, pulp regeneration.

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**Introduction**

RET are becoming widely used in the management of immature permanent teeth with necrotic pulp tissues, in which other alternative treatment options offer less successful outcomes (Galler, 2016). RET are biologically based treatments that aim to replace damaged tissue structures, including the pulpdentine complex (Murray et al., 2007). This treatment strategy, if successful, could potentially allow the continuation of root maturation, therefore, improving the long-term prognosis of these compromised teeth (Conde et al., 2016; Garcia-Godoy and Murray, 2012). Clinically this treatment involves no or very minimal root canal instrumentation, disinfection, followed by induction of apical bleeding into the root canal space (Banchs and Trope, 2004; Iwaya et al., 2001). Due to the limited or no mechanical instrumentation, microbial elimination is mainly dependent on antimicrobial agents for sufficient canal disinfection. Despite remarkable progress in the field of RET over the past decade, several challenges remain (Tong et al., 2017), such as the questionable efficacy of the currently available disinfection techniques in promoting continuation of root development (Fouad, 2020).

Furthermore, RET is a stem cell-based regeneration strategy. Hence the balance between root canal disinfection and keeping the microenvironment as hospitable as possible for the stem cells to regenerate the pulp-dentine complex is of prime importance (Kim et al., 2018). Many of the currently used intra-canal irrigants such as NaOCl and CHX were proven to have a cytotoxic effect on stem cells (Martin et al., 2014; Widbiller et al., 2019). The use of topical antibiotic pastes are effective endodontic antimicrobials; however, in addition to tooth discoloration caused by some of the antibiotic combinations (Lenherr et al., 2012), a level of cytotoxicity on stem cells in a concentration-dependent manner is of clinical concern (Ruparel et al., 2012). Although using topical antibiotic pastes at lower concentrations provided adequate canal disinfection, such mixtures are difficult to prepare and obtain. The argument about their contribution to the global challenge of antimicrobial resistance at such low concentrations is yet to be settled (Ayoub et al., 2020; Yadlapati et al., 2017; Yadlapati et al., 2014).

Therefore, the development of alternative disinfectant strategies with sufficient antimicrobial efficacy, biocompatibility, and regenerative potential (with the least harm to the surrounding conductive microenvironment) is of importance in this field. The achievement of a safer and more predictable regenerative outcome is clinically required (Diogenes et al., 2014; Fouad, 2020). With the current advances in tissue engineering, this gap is being addressed through several innovative natural and synthetic strategies, such as alternative antimicrobial agents, nano-based delivery systems, and novel photosensitisers combined with PDT which initiate tissue regeneration and possess an innate antimicrobial activity (Chung and Park, 2017; Samiei et al., 2016). A schematic illustration depicting limitations of currently used antimicrobial strategies and the potential benefits of alternative RET antimicrobial strategies is provided (Fig. 1).

Taking all of the above into consideration, it seems comprehensible to develop an alternative antimicrobial able to disinfect the root canal system without compromising the regenerative environment. The aim of this review is to critically discuss and challenge the current status quo of antimicrobials
used in regenerative endodontics, and to shed the light on future alternative antimicrobial materials with regenerative potential.

**Literature search and scope of the review**

An electronic search of PubMed and Elsevier's Scopus was undertaken with appropriate MeSH terms and various keyword combinations including “antimicrobial”, “antibiotic”, “disinfection”, “dentistry”, “pulp revascularisation”, “pulp regeneration”, “regenerative endodontic”, “drug delivery”, “biomaterials”, and “dental pulp stem cells”. The reference list of the relevant articles resulting from database searches was further hand-screened. No limits were applied to the year of publication and only English language literature was included. However, due to the scope and extent of this search, a wide-ranging comprehensive narrative review of antimicrobial strategies in regenerative endodontics rather than a systematic review was undertaken.

**Current antimicrobial strategies used in RET**

A critical summary of the most commonly used disinfectant agents (irrigants and medicaments) in terms of their antimicrobial efficacy and biocompatibility will be discussed below as these have been extensively reviewed in the literature (Diogenes et al., 2014; Kim et al., 2018; Martin et al., 2014).

**Intra-canal irrigation**

NaOCl is one of the oldest endodontic irrigants and reported in most published RET studies, albeit in various concentrations ranging from 1 – 6 % (Tong et al., 2020). NaOCl solution is regarded as the irrigant of choice mainly due to its bacteriostatic, bactericidal, and tissue dissolution properties (Bryce et al., 2009; Zehnder, 2006). The ability of NaOCl to dissolve organic tissue is well-documented, as well as its negative effects on the mechanical properties of root dentine (Dotto et al., 2020; Pascon et al., 2009). These mechanical alterations are most likely due to the proteolytic action of concentrated hypochlorite solution on the collagen dentine matrix (Zehnder, 2006). Alterations in the dentine-matrix composition including the denaturation of growth factors and attachment proteins are likely to affect the fate of stem cells (Diogenes et al., 2014). Furthermore, NaOCl at a concentration between 5-6 %, has shown detrimental effects on stem cell numbers and survival as well as loss of odontoblast-like phenotype differentiation both in vitro and in vivo (Casagrande et al., 2010; Galler et al., 2011).

From a biological perspective, a concentration-dependent effect of NaOCl on the survival of SCAP has been shown, with 6 % NaOCl showing the greatest reduction in stem cell survival. This resulted in the recommendation for using a low NaOCl concentration of 1.5 % (Web ref.1; Martin et al., 2014; Trevino et al., 2011). However, controversies remain in terms of the ability of low NaOCl concentrations to completely eradicate infected biofilms (Ma et al., 2015; Tagelsir et al., 2016). In an attempt to reduce such effect, 17 % EDTA has been recommended following the use of NaOCl irrigation. This step has been shown to reduce NaOCl detrimental side effects on stem cell survival, regardless of the NaOCl concentration used (Martin et al., 2014; Trevino et al., 2011). Furthermore, combining EDTA within a given irrigation protocol was also found to significantly increase the release of growth factors such as TGF β-1 into the root canal space, hence inducing DPSCs migration and differentiation to odontoblasts (Zeng et al., 2016).

CHX is also one of the well known endodontic disinfectant agents used in RET and clinically available in the form of an aqueous solution or gel preparation with a dilution range of 0.12 % to 2 % (Okino et al., 2004). However, the use of this irrigant is less prevalent within RET protocols as highlighted in a recent survey, in which only 11.4 % of respondents used CHX as the sole disinfectant, while 22.2 % reported a combined use of CHX and NaOCl (Tong et al., 2020). CHX benefits from broad-spectrum antimicrobial and intra-canal substantivity (residual effect) properties (Martin et al., 2014; Okino et al., 2004; Trevino et al., 2011). The use of 2 % CHX has also been shown to cause unfavourable effects on stem cell survival (Trevino et al., 2011) and attachment (Ring et al., 2008), with direct cytotoxicity effect in a concentration-dependent manner (Widbiller et al., 2019). On the contrary, CHX lacks organic tissue dissolution ability (Okino et al., 2004); therefore, its effect on biofilm disruption is questioned (Bryce et al., 2009; Trevino et al., 2011).

**Intra-canal medicaments**

Intra-canal medicaments are commonly used interappointment antimicrobial RET dressing (Tong et al., 2020) and broadly divided into two groups; topical antibiotic pastes or Ca(OH)₂. Topical antibiotic pastes used within RET mainly include; TAP (ciprofloxacin, metronidazole, and minocycline), DAP (ciprofloxacin and metronidazole), and other modified formulations (Tong et al., 2020). Although sufficient antimicrobial efficacy is one of the main advantages behind the use of topical antibiotic pastes, clinical limitations have been raised (Ribeiro et al., 2020).

Complete removal of the applied antibiotic paste is crucially important for a successful regenerative outcome and to avoid unwanted, possibly long-term, side effects. Unfortunately, studies have demonstrated that a significant amount of antibiotic pastes (88 % residual) remains within the root canal system following current irrigation techniques (Berkhoff et al., 2014). Indirect negative effects, such as reduction in dentinal strength and fracture resistance, are reported as early as 1 week post-
application. These effects were mainly linked to the strong demineralisation effect and the acidic nature of antibiotic pastes on the surrounding dentine matrix (Yassen et al., 2013a; Yassen et al., 2013b). These structural changes are thought to affect the fate of stem cells and hinder their regenerative potential, consequently resulting in inconsistent clinical results related to continuation of root maturation, thickening of root dentine, and apical closure (Tong et al., 2017).

A concentration-dependent detrimental effect of various topical antibiotic pastes on human SCAP survival has been shown in vitro (Althumairy et al., 2014; Ruparel et al., 2012). Therefore, to achieve optimal results, various antibiotic mixtures and concentrations have been tested. A low TAP and DAP concentration of 1 mg/mL provided sufficient antimicrobial efficacy with no reported negative effects on the viability of SCAP when compared to higher concentrations (1,000 mg/mL) (Althumairy et al., 2014). Furthermore, the potential development of bacterial resistance biofilms and/or sensitisation has been raised (Berkhoff et al., 2014; Stewart and William Costerton, 2001). Coronal discolouration is also a commonly reported side effect of the TAP use, which is largely linked to minocycline, a semisynthetic tetracycline antibiotic (Kim et al., 2010; Lenherr et al., 2012; Sato et al., 1996). However, despite omitting minocycline within RET protocols tooth discoloration continued to be reported (Tong et al., 2017).

Ca(OH)$_2$ is another widely used intra-canal medicament advocated to overcome the undesirable effects of the topical antibiotic pastes and recommended by the European Society of Endodontology for short-term clinical application (Galler et al., 2016). Material advantages such as availability and ease of removal from the root canal are documented (Berkhoff et al., 2014; Nazzael et al., 2018).

Nevertheless, conflicting antimicrobial efficacy of Ca(OH)$_2$ as an intra-canal dressing, has been reported (Ribeiro et al., 2020). The ability of Ca(OH)$_2$ to eradicate specific bacteria, such as E. faecalis, and yeasts from the root canal systems has been questioned (Krithikadatta et al., 2007; Zehnder et al., 2004). More recently, a clinical molecular-based study showed comparable antimicrobial efficacy and regenerative outcome following the use of TAP and a combined Ca(OH)$_2$/CHX paste (de-Jesus-Soares et al., 2020). From a biological perspective, Ca(OH)$_2$ provided an environment conducive to stem cell survival and proliferation (Althumairy et al., 2014; Ruparel et al., 2012). However, the possible side effects of Ca(OH)$_2$ on the biological property of dentine-matrix-derived growth factors have been highlighted and requires consideration (Kim et al., 2018).

Possible alternative antimicrobial strategies with a regenerative potential for RET

The development and characterisation of the next generation novel materials that can enhance the regeneration of pulp-dentine complex as well as provide sufficient antimicrobial properties is a recent focus area for a safer and predictable regenerative outcome (Chung and Park, 2017). Indeed, a recent scoping review of the literature concluded that trends towards alternative antimicrobials are promising and deserve future consideration (Ribeiro et al., 2020). Word cloud highlighting of both natural and synthetic alternative antimicrobial materials and strategies for potential use in RET was performed. The more a substance has been researched, within this specific field, the larger it appears in the word cloud (Fig. 2).
Natural materials and strategies

Propolis
Propolis, also known as “bee glue”, is a natural resinous substance crucial for both internal and external beehive protection (Ghisalberti, 1979; Grange and Davey, 1990). This resinous material is initially collected by bees (Apis mellifera) from exudates and plant buds, subsequently mixed with saliva secretions (bee enzymes) and wax (Ghisalberti, 1979; Grange and Davey, 1990). Historically, propolis has been used by on Egyptian, Greek and Roman traditional medicine since 300 BC (Khalil, 2006; Sforcin and Bankova, 2011).

Propolis comprises mainly resins, balsams, and wax in addition to amino acids, aromatic compounds, phenols, pollens, minerals, and vitamins (Ghisalberti, 1979; Grange and Davey, 1990; Uzel et al., 2005). The resinous portion is mainly composed of flavonoid pigments (well-known plant compounds) and is regarded the main active component, linked to propolis broad-spectrum antimicrobial activity (Ghisalberti, 1979; Grange and Davey, 1990). Additional therapeutic features include anti-oxidant, anti-cariogenic, and anti-inflammatory properties (Khalil, 2006; Marcucci, 1995; Uzel et al., 2005).

This resinous material has been highlighted as a promising natural additive to the chemical composition of toothpastes (Morawiec et al., 2013) and mouthwashes (Dodwad and Kukreja, 2011; Halboub et al., 2020). Possible avenues of propolis use in dentistry include prevention of dental caries and plaque formation (Koo et al., 2020). Possible avenues of propolis use in dentistry include prevention of dental caries and plaque formation (Koo et al., 2020). Propolis, at concentrations of 4 mg/mL or lower, was found to be at least 10 times less cytotoxic to fibroblasts of the dental pulp and periodontal ligament when compared with Ca(OH)₂ (Al-Shaher et al., 2004). The use of propolis as a vehicle for Ca(OH)₂ has also been suggested, with in vitro studies concluding efficient diffusion throughout the dentinal tubules, and possibly extending to the external root surface (Baranwal et al., 2017; Montero and Mori, 2012; de Rezende et al., 2008). Furthermore, animal studies utilising propolis paste as an intra-canonical medicament have shown promising results equal to or superior to TAP in terms of soft and hard tissue deposition (El-Tayeb et al., 2019; Pagliarin et al., 2016). In vitro and in vivo studies utilising propolis as an intra-canonical disinfectant agent are summarised in Table 1 and 2, respectively.

Chitosan-based particles
Chitosan (poly[1,4]-b-D-glucopyranosamine) is a cationic natural nontoxic biopolymer obtained by the alkaline deacetylation of chitin (Peter, 1995; Rabea et al., 2003). Chitin is the second most abundant natural polymer found in the exoskeleton of marine crustaceans such as shrimps and crab shells (Peter, 1995; Rabea et al., 2003).

Chitosan has a high nitrogen content with a strong chelating ability and great commercial interest (Rabea et al., 2003). On a production scale, chitosan can be produced in several forms, such as a paste or powder, with particles at the macro- or nano-scale (Agnihotri et al., 2004). Commercially, chitosan is available with an average molecular weight of 3,800 - 20,000 Daltons and 66 - 95 % deacetylation (Agnihotri et al., 2004). Its versatile commercial applications include environmental, agricultural, food additive, and a hydrating agent in cosmetics (Peter, 1995; Rabea et al., 2003). Chitosan-based particles are regarded as an effective drug delivery system (Li et al., 2018), and tested as a vehicle for Ca(OH)₂ or TAP with promising results such as improved stability and promoting a sustained release of medicament (Ballal et al., 2010; del Carpio-Perochena et al., 2017; Shaik et al., 2014). More recently, chitosan has been explored as an antimicrobial agent to disinfect the root canal system with proposed regenerative potential (Ducret et al., 2019; Palma et al., 2017).

This attractive research interest is linked to its unique biological properties such as biocompatibility, excellent bioadhesive, and broad-spectrum antimicrobial properties (Raafat and Sahl, 2009; Rabea et al., 2003; Shrestha et al., 2010). A postulated
mechanism of chitosan antimicrobial activity is linked to the interaction between positively charged chitosan (\(\text{NH}_3^+\) groups of glucosamine) and negatively charged cell membrane causing a sequential of events, which alter the cell permeability and consequently cell death (Raafat and Sahl, 2009; Rabea et al., 2003). Additional advantages for extended clinical applications include abundance in nature, ease of modification, nontoxic, and low production cost (Peter, 1995; Rabea et al., 2003).

However, in spite of chitosan’s proven biocompatibility and noncytotoxicity (Renard et al., 2020), its regenerative potential in dentistry remains controversial. Preliminary in vitro work demonstrated stimulation of dental pulp tissue formation, in terms of mesenchymal stem cells viability and deposition of dental pulp-like collagen matrix following the use of a novel chitosan-enriched fibrin hydrogel (Ducret et al., 2019). In-contrast, the clinical application of chitosan scaffolds in immature dog teeth with apical periodontitis resulted in no histologic evidence of pulp-dentine tissue regeneration nor newly formed mineralised tissue (Palma et al., 2017). The degradation process of chitosan under inflammatory conditions requires careful assessment prior to clinical translation (Palma et al., 2017; Yamada et al., 2014). In vitro studies utilising chitosan-based particles as an intra-canal disinfectant agent are summarised in Table 1.

Table 1a. In vitro and ex vivo studies of alternative antimicrobial strategies highlighting antimicrobial type, origin, study design, usage, study and control groups, assessment method and duration, microorganisms tested and main findings.

<table>
<thead>
<tr>
<th>Alternative antimicrobial/origin</th>
<th>Study design/usage</th>
<th>Study groups</th>
<th>Control groups</th>
<th>Microorganism</th>
<th>Assessment method/duration</th>
<th>Main findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propolis/ Nature Home, Amman, Jordan</td>
<td>Human dentine block model Medicament</td>
<td>30% propolis Ca(OH)(_2)</td>
<td>Saline Sterile uninoculated broth</td>
<td>E. faecalis</td>
<td>Microbiological samples were collected (paper points, headstrom files and disc immersion), incubated on agar plates and CFUs analysed</td>
<td>Propolis was significantly more effective than Ca(OH)(_2) for short-term application</td>
<td>Awwadheh et al., 2009</td>
</tr>
<tr>
<td>Propolis</td>
<td>Human dentine block model Irrigant</td>
<td>Propolis MCJ 2% POVI 2% CHX gel Ca(OH)(_2)</td>
<td>Saline</td>
<td>E. faecalis</td>
<td>Dentine shavings were collected (200 and 400 µm depths), cultured on TS agar plates and CFUs analysed</td>
<td>CHX produced the highest antimicrobial efficacy followed by POVI, propolis and MCJ. Ca(OH)(_2) was least effective</td>
<td>Kandaswamy et al., 2010</td>
</tr>
<tr>
<td>Propolis/ Apis flora, Ribeirão Preto, Brazil</td>
<td>Human root model Irrigant</td>
<td>12% propolis glycolic extract</td>
<td>Saline</td>
<td>E. coli</td>
<td>Microbiological samples were collected, incubated on agar plates and CFUs analysed</td>
<td>Propolis was effective to completely eliminate (E.) coli and reduce the amount of endotoxins</td>
<td>Valera et al., 2010</td>
</tr>
<tr>
<td>Propolis/ Turkey (northeast and northwest areas)</td>
<td>Human dentine block model Irrigant</td>
<td>EEP 2% CHX solution Ca(OH)(_2) 96% ethanol PBS</td>
<td>Saline</td>
<td>E. faecalis</td>
<td>Dentine shavings were collected (300 µm depth), cultured on TS agar plates and CFUs analysed</td>
<td>Propolis antimicrobial efficacy was higher than Ca(OH)(_2) and lower than CHX</td>
<td>Kayaoglu et al., 2011</td>
</tr>
<tr>
<td>Propolis/ Calgary gold bee products, Canada</td>
<td>Human root model Medicament</td>
<td>Ca(OH)(_2) TAP EEP ethanol</td>
<td>Saline</td>
<td>E. faecalis</td>
<td>Percentage reduction in colony counts</td>
<td>Propolis was more effective than TAP at day 2 and equally effective at day 7</td>
<td>Madhubala et al., 2011</td>
</tr>
</tbody>
</table>
Curcumin
Curcumin (diferuloylmethane), a dimeric derivative of ferulic acid, is the main bioactive substance of C. longa (turmeric), a wellknown oriental spice (Adamczak et al., 2020). Curcumin pigment, a distinctive yellow-orange colouring matter of plant origin, was isolated back in 1842 by Vogel and Pelletier from the rhizomes of C. longa, originating from the ginger family tree native to South Asia (Adamczak et al., 2020; Hewlings and Kalman, 2017). Curcumin possesses a wide spectrum of bioactive and therapeutic properties such as antibacterial, antifungal, and antiviral properties (Praditya et al., 2020; Hewlings and Kalman, 2017). Antioxidant and anti-inflammatory activities are also documented in the literature (Hewlings and Kalman, 2017; Rai et al., 2020). The antimicrobial action of curcumin is attributed to their ability to damage the bacterial cell membrane through the suppression of bacterial cytokinesis, the induction of filamentation, and the inhibition of the FtsZ assembly dynamics in the Z-ring (Rai et al., 2008). Inhibition of cellular proliferation and alterations of gene expression are also reported to be behind the bactericidal mechanisms of curcumins (Rai et al., 2008; Rai et al., 2020; Tyagi et al., 2015).

In the past, the clinical usage of curcumins was limited due to their poor water solubility, low oral bioavailability, and rapid metabolism (Chang et al., 2018; Sinjari et al., 2019). The use of liposomes, in order to solubilise curcumin phospholipidic bilayer, has been suggested to enhance curcumin delivery and improve its therapeutic efficiency (Chang et al., 2018; Sinjari et al., 2019). Sinjari et al. (2019) closely assessed the direct contact of human DPSCs with nanocarrier curcumin-loaded tropoelastin and also studied the influence of biomaterials on the proliferation of human DPSCs with nanoparticle curcumin-loaded tropoelastin.

Table 1b. In vitro and ex vivo studies of alternative antimicrobial strategies highlighting antimicrobial type, origin, study design, usage, study and control groups, assessment method and duration, microorganisms tested and main findings.

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<th>Main findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propolis/ Beehives of Najaf Abad, Esfahan</td>
<td>Human root model Medicament</td>
<td>EEP Ca(OH)$_2$ ethanol</td>
<td>No medicament Sterile samples</td>
<td>E. faecalis</td>
<td>Microbiological samples were collected with a piezoreamer, plated and CFU analysed. MIC was also measured using dilution methods</td>
<td>7 d</td>
<td>Zare Jahromi et al., 2012</td>
</tr>
<tr>
<td>Propolis/ RK’s Aroma Products, Mumbai</td>
<td>Human root model Medicament</td>
<td>Propolis 2 % CHX gel Ca(OH)$_2$</td>
<td>Saline E. faecalis</td>
<td>Dentine shavings were collected (depth of 400 µm), cultured on TS agar and CFUs analysed</td>
<td>1, 3 and 5 d</td>
<td>Propolis had greater antimicrobial efficacy than Ca(OH)$_2$, on day 1, with no significant difference in subsequent days</td>
<td>Bhandari et al., 2014</td>
</tr>
<tr>
<td>Propolis/ Natural Bee Health Industry, Lima, Peru</td>
<td>Human root model Irrigant</td>
<td>Ca(OH)$_2$ Propolis 2 % CHX gel</td>
<td>Saline E. faecalis and C. albicans</td>
<td>Dentine shavings were collected (100 and 200 µm depths), cultured on agar blood or agar Sabouraud plates and CFUs analysed</td>
<td>14 d</td>
<td>Propolis and CHX were the most effective against E. faecalis. However, CHX had the highest antifungal activity</td>
<td>Carbajal Mejia, 2014</td>
</tr>
<tr>
<td>Propolis/ Stakich, Royal Oak, Michigan, USA</td>
<td>Human root model Medicament</td>
<td>95 % propolis TAP 2 % CHX gel Ca(OH)$_2$</td>
<td>Saline C. albicans</td>
<td>Dentine shavings were collected (200 and 400 µm depths) and CFUs analysed</td>
<td>1 and 7 d</td>
<td>Propolis was less effective on day 1 and equally effective to other medicaments on day 7</td>
<td>Chua et al., 2014</td>
</tr>
<tr>
<td>Propolis/ Herbal Biosolutions, Delhi C. longa/ RYM exports, Mumbai, India</td>
<td>Human dentine block model Medicament</td>
<td>2 % CHX gel honey Aloe vera gel 20 % C. longa gel 11 % EEP Ca(OH)$_2$</td>
<td>Saline E. faecalis</td>
<td>Dentine shavings were collected (200 and 400 µm depths) and CFUs were analysed.</td>
<td>1, 3 and 5 d</td>
<td>2 % CHX gel was most effective followed by propolis and C. longa</td>
<td>Vasudeva et al., 2017</td>
</tr>
</tbody>
</table>
liposome in the presence of hydrophilic monomers (2-hydroxyethyl methacrylate). Quantitative cytokine release assessment showed a decreased secretion of tested pro-inflammatory cytokines (interleukin 6, interleukin 8, interferon-gamma, and monocyte chemoattractant protein-1), in response to curcumin liposome nanocarriers. The authors concluded that curcumin liposome nanocarriers had stimulated DPSCs proliferation and reduced inflammation via the NFkB/ERK/pERK pathways, but did not induce odontoblastic differentiation (Sinjari et al., 2019).

Various loading and encapsulation mechanisms for curcumin delivery such as nanoemulsion, nanosuspension, lipid nanoparticles, and hydrogel nanoparticles were further investigated in the literature (Dutta and Ikiki, 2013; Rai et al., 2020).

Despite the above-mentioned therapeutic and bioactive properties, limited studies were conducted within the dental field, particularly in RET (Neelakantan et al., 2013; Yadav et al., 2018). Emerging in vitro studies, with promising results, expanding the use of curcumin as an intra-canal disinfectant agent are summarised in Table 1.

**AgNPs**

Inorganic metals, in their standard or ionic forms such as Ag or Ag⁺, are regarded as antibiotic alternatives due to their broad-spectrum bactericidal effects coupled with the unlikely possibility of developing antibiotic-resistant bacterial strains (Oei et al., 2012; Rai et al., 2009). Specifically, AgNPs have gained recent popularity owing to their distinctive physical and biochemical properties (Bapat et al., 2018; Rai et al., 2009), synergistic antibiotic effect (Fayaz et al., 2010), biocompatibility (Franková et al., 2016; Gomes-Filho et al., 2010), and antimicrobial properties (Lara et al., 2010; Rai et al., 2009). The ability of AgNPs to disrupt dental biofilms and prevent bacterial adhesion are also advantageous (Wu et al., 2014).

The incorporation of silver particles within dental materials is not new and has been practiced for decades since the use of silver-containing dental amalgam (Noronha et al., 2017). However, with advanced nanotechnology, AgNPs have gained considerable attention (Bapat et al., 2018; Noronha et al., 2017). The incorporation of AgNPs within a diverse range of dental materials have been proposed, such as composite resins (Cheng et al., 2012; Durner et al., 2011), dental implant coating (Cao et al., 2011; Wang et al., 2013), calcium silicates cements (Fan et al., 2014; Samiei et al., 2013), endodontic sealers (Vilela Teixeira et al., 2017), and intra-canal disinfectant agents (Afkhami et al., 2017; Afkhami et al., 2015; Moazami et al., 2018; Wu et al., 2014).

The antimicrobial mode of action of AgNPs is largely associated with the release of Ag⁺ ions, which consequently penetrate the cell membrane and interact with various cellular components, resulting in inhibition of cell replication and eventually cell death (Bapat et al., 2018; Rai et al., 2009). An immediate

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**Table 1c. In vitro and ex vivo studies of alternative antimicrobial strategies highlighting antimicrobial type, origin, study design, usage, study and control groups, assessment method and duration, microorganisms tested and main findings.**

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<th>Main findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan/ Sigma-Aldrich Inc.</td>
<td>Well plates</td>
<td>Chitosan-nanoparticle/Zinc oxide-nanoparticle</td>
<td>-</td>
<td>2 strains of E. faecalis in planktonic and biofilm forms</td>
<td>LIVE/DEAD staining/confocal microscopy/CFUs 90 d</td>
<td>The incorporation of nanoparticles enhanced antimicrobial efficacy with retained aging potential</td>
<td>Shrestha et al., 2010</td>
</tr>
<tr>
<td>Chitosan/ Acros Organics</td>
<td>Human root model</td>
<td>15 % EDTA 0.2 % chitosan 10 % citric acid 1 % acetic acid</td>
<td>No final irrigation</td>
<td>-</td>
<td>SEM/ atomic absorption spectrophotometry</td>
<td>0.2 % chitosan was similar to 15 % EDTA in terms of smear layer and dentine demineralisation</td>
<td>Silva et al., 2013</td>
</tr>
<tr>
<td>Chitosan/ Mahtani Chitosan Pvt. Ltd Veraval, India</td>
<td>Human root model</td>
<td>0.25 % chitosan 0.5 % chitosan 2 % CHX 3 % NaOCl</td>
<td>Saline</td>
<td>E. faecalis and C. albicans biofilms</td>
<td>Agar-well diffusion method/MIC/biofilm susceptibility assay/SEM/ cytotoxicity assay/CFUs</td>
<td>Similar antimicrobial efficacy was seen between study groups. However, chitosan showed significant less toxicity than NaOCl</td>
<td>Yadav et al., 2017</td>
</tr>
<tr>
<td>Curcumin/ Biopurify Phytochemicals Ltd, Sichuan, China</td>
<td>Human root model</td>
<td>Curcumin 2 % CHX 3 % NaOCl</td>
<td>PBS</td>
<td>E. faecalis biofilms</td>
<td>MIC/ MBC/CFUs 2&lt;sup&gt;nd&lt;/sup&gt; d, 2&lt;sup&gt;nd&lt;/sup&gt; and 8&lt;sup&gt;th&lt;/sup&gt; weeks</td>
<td>Curcumin antimicrobial efficacy was similar to 3 % NaOCl at 2&lt;sup&gt;nd&lt;/sup&gt; and 2&lt;sup&gt;nd&lt;/sup&gt; and 8&lt;sup&gt;th&lt;/sup&gt; weeks and inferior to CHX at 8&lt;sup&gt;th&lt;/sup&gt; week</td>
<td>Neelakantan et al., 2013</td>
</tr>
</tbody>
</table>
bactericidal antimicrobial effect of AgNPs has been shown against several drug-resistant and drug-susceptible bacteria possibly through inhibiting cell wall, protein, and nucleic acid synthesis (Lara et al., 2010).

However, there are some cytotoxicity concerns to the use of AgNPs, mainly related to adverse events of free Ag⁺. Such concerns are controversial and at an early stage of research for sound conclusions to be drawn. Franková et al. (2016) assessed AgNPs against human dermal fibroblasts and epidermal keratinocytes. They concluded that AgNPs inhibit the production of pro-inflammatory cytokines and contributed positively towards the wound-healing process. Quantitative elution testing and qualitative on-growth of human osteoblasts also revealed no cytotoxicity following the use of 1% nano-silver loaded bone cement in vitro (Alt et al., 2004). Indeed, it was proven that AgNPs biocompatibility is concentration dependent with increased cytotoxicity at higher concentrations (Gomes-Filho et al., 2010; Newby et al., 2011). In addition to material concentration, material-specific characteristics (Cao et al., 2010; Newby, 2011). In addition to material origin, study design, usage, study and control groups, assessment method and duration, microorganisms

Argyria, an irreversible skin pigmentation, is a well-known silver-related side effect (Drake and Hazelwood, 2005). Furthermore, potential tooth discoloration is also of concern and requires careful investigation prior to clinical usage (Afkhami et al., 2015). In vitro spectrophotometric analysis showed no significant colour changes between AgNPs added to Ca(OH)₂ compared to Ca(OH)₂ alone when used as an intra-canal medicament (Afkhami et al., 2017). In contrast, spectrophotometric analysis of AgNPs Table 1d. In vitro and ex vivo studies of alternative antimicrobial strategies highlighting antimicrobial type, origin, study design, usage, study and control groups, assessment method and duration, microorganisms tested and main findings.

<table>
<thead>
<tr>
<th>Alternative antimicrobial/origin</th>
<th>Study design/usage</th>
<th>Study groups</th>
<th>Control groups</th>
<th>Microorganism</th>
<th>Assessment method/duration</th>
<th>Main findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photoactivated curcumin/20μmol/L</td>
<td>Human root model irrigant</td>
<td>Curcumin</td>
<td>Physiological solution</td>
<td>E. faecalis biofilms</td>
<td>Fibre optic LED of 100 mW/cm²</td>
<td>Photoactivated curcumin reduced, however did not eliminate, bacterial count</td>
<td>da Frota et al., 2015</td>
</tr>
<tr>
<td>Sigma-Aldrich, St Louis, MO, USA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Light duration of 5 and 10 min was investigated CFUs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Curcumin/2.5 mg/mL</td>
<td>Human root model irrigant</td>
<td>Curcumin 3% NaOCl</td>
<td>Saline</td>
<td>E. faecalis biofilms</td>
<td>Ultrasonic activated for 30 s cycles for 4 min/ photoactivated with blue light of 1200 mW/cm² for 4 min/SEM CFUs</td>
<td>Photoactivated curcumin produced the maximum elimination of biofilm bacteria</td>
<td>Neelakantan et al., 2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Before treatment, immediately after treatment, and after 7 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Photoactivated curcumin/2.5 mg/mL</td>
<td>Human root model</td>
<td>Photoactivated curcumin TAP DAP 2% CHX Ca(OH)₂</td>
<td>No medicament</td>
<td>E. faecalis biofilm</td>
<td>LIVE/DEAD staining/CLSM/CFUs</td>
<td>Photoactivated curcumin produced superior antimicrobial and antibiofilm activity</td>
<td>Devaraj et al., 2016</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Curcumin/2.5 and 5.0 mg/mL</td>
<td>Well plates irrigant</td>
<td>Curcumin-electrospun modified fibres</td>
<td>Saline</td>
<td>A. naeslundii biofilms</td>
<td>CFUs</td>
<td>Photoactivated curcumin-based medicaments showed a high antibiofilm activity when used at low concentrations</td>
<td>Sotomil et al., 2019</td>
</tr>
</tbody>
</table>

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coated with imidazolium as a root canal irrigant resulted in statistically significant darker colour changes in comparison with the control groups (5.25 % NaOCl, 2 % CHX, and normal saline) (Moazami et al., 2018). The colour change caused by AgNPs could be linked mostly to the direct contact of the irrigant material with the coronal dentine, which is practically difficult to control during irrigation. Taking the above results into consideration, well-controlled clinical studies are warranted for sound conclusions and clinical recommendations. Emerging studies utilising AgNPs within the endodontic field as an intra-canal disinfectant agent are summarised in Table 1.

### Graphene-based materials

Graphene is a recently developed material made of densely packed carbon atoms arranged as a two-dimensional monolayer sheet forming a benzene hexagonal structure (Novoselov et al., 2004). Examples of graphene derivatives include pristine graphene, graphene oxide, reduced graphene oxide, few-layered graphene, ultrathin graphite, graphene nanosheets, and graphene-based nanocomposites (Guazzo et al., 2018; Tahiri et al., 2019). These different derivatives mainly differ in the material size, number of layers, and specific surface characteristics (Guazzo et al., 2018).

Graphene and its derivatives have a wide range of biomedical applications due to their unique mechanical, electrochemical, and physical properties such as mechanical strength, high surface area, thermal stability, and unique surface characteristics (Guazzo et al., 2018; Tahiri et al., 2019). Furthermore, their ability to combine and functionalise with various materials and bioactive molecules to enhance or alter specific properties for distinctive applications is of interest (Guazzo et al., 2018).

Graphene-based materials also have broad-spectrum, yet variable, antimicrobial properties (Al-Jumaili et al., 2017; Guazzo et al., 2018). Its antimicrobial mode of action is explained through different mechanisms.

1. A sharp knife-edge cutting effect that consequently leads to cell membrane rupture, mechanical stress induction, and phospholipids extraction.

### Table 1. In vitro and ex vivo studies of alternative antimicrobial strategies highlighting antimicrobial type, origin, study design, usage, study and control groups, assessment method and duration, microorganisms tested and main findings.

<table>
<thead>
<tr>
<th>Alternative antimicrobial/ origin</th>
<th>Study design/ usage</th>
<th>Study groups</th>
<th>Control groups</th>
<th>Microorganism</th>
<th>Assessment method/ duration</th>
<th>Main findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. longa extract/ Curenex, Abbott, India</td>
<td>Human root model Medicament</td>
<td>C. longa extract</td>
<td>-</td>
<td>E. faecalis biofilms</td>
<td>CHX gel showed highest antimicrobial efficacy followed by C. longa extract, while Ca(OH)₂ was least effective</td>
<td>Yadav et al., 2018</td>
<td></td>
</tr>
<tr>
<td>Nano-silver liquid form/ Nanocid Company, Tehran, Iran</td>
<td>Test tubes and inoculated plates Irritant</td>
<td>Nano-silver 2 % CHX 5.25 % NaOCl</td>
<td>Vancomycin bacterial susceptibility papers Saline</td>
<td>E. faecalis</td>
<td>MIC/ agar diffusion/ zones of inhibition 6, 18, 24 and 48 h of incubation</td>
<td>Nano-silver showed equal bactericidal action as 5.25 % NaOCl</td>
<td>Lotfi et al., 2011</td>
</tr>
<tr>
<td>Silver nitrate/ Factory of Shanghai Chemical Reagent Ltd, China</td>
<td>Longitudinal split root human teeth model</td>
<td>Nano-silver incorporated mesoporous calcium silicate Ca(OH)₂ deionised water</td>
<td>E. faecalis</td>
<td>LIVE/DEAD staining/ cell counting kit-8 Variable</td>
<td>The incorporation of Ag particles resulted in enhanced antibacterial efficacy and reduced bacterial colonisation</td>
<td>Fan et al., 2014</td>
<td></td>
</tr>
<tr>
<td>AgNPs/ Huzheng Nano Technology Ltd, Shanghai, China</td>
<td>Longitudinal split root human teeth model Irritant/ medicament</td>
<td>Stage 1; 0.1 % AgNPs solution 2 % NaOCl Stage 2; 0.01 % AgNPs gel 0.02 % AgNPs gel Ca(OH)₂</td>
<td>Sterile saline</td>
<td>E. faecalis</td>
<td>SEM/ LIVE/DEAD staining / CLSM Stage 1; for 2 min Stage 2; for 7 d</td>
<td>0.02 % AgNPs gel as a medicament resulted in significant reduction in residual biofilms</td>
<td>Wu et al., 2014</td>
</tr>
<tr>
<td>Ag-GO</td>
<td>Human root model Irritant</td>
<td>1 % NaOCl 2.5 % NaOCl 2 % CHX 17 % EDTA 0.25 % Ag-GO</td>
<td>Saline</td>
<td>P. acnes, A. radicidentis, S. epidermidis, and S. mitis Paper point sampling/ microbial counts/ CLSM</td>
<td>Ag-GO resulted in significant bacterial reduction, however, 2.5 % NaOCl showed highest antimicrobial efficacy</td>
<td>Ioannidis et al., 2019</td>
<td></td>
</tr>
</tbody>
</table>
from the cell membrane (Al-Jumaili et al., 2017; Zhou and Gao, 2014)

2. Cell entrapment. Aggregated graphene sheets can trap and separate microorganisms from their microenvironment. This environmental disconnection results in preventing nutrient consumption, reducing proliferative activity, and eventually inactivation of the microorganism (Akhavan et al., 2011)

3. Oxidative stress induction can also act as an electron pump transferring electrons out of microorganisms (Liu et al., 2011).

However, the exact antimicrobial effectiveness is difficult to predict and is thought to be dependent on several factors such as type of microorganisms, duration of exposure, and most importantly specific structural properties – such as number of layers, porosity, size, and shape of the individual graphene sheets (Li et al., 2014; Liu et al., 2011; Zhu et al., 2015).

In general, graphene-based materials are biocompatible, promoting cell adhesion, proliferation, and differentiation (Menaa et al., 2015; Tahriri et al., 2019). However, the exact degree of biocompatibility and potential cytotoxicity is highly dependent on their oxygen functional groups (Guazzo et al., 2018).

Despite the limited in vivo studies, in vitro studies of variable methodologies concluded that most graphene-based hydrophilic forms are less toxic than the hydrophobic forms (Guazzo et al., 2018; Tahriri et al., 2019).

To further investigate graphenes’ biocompatibility and and dental tissue regeneration, Rosa et al. (2016) investigated the proliferation and differentiation potential of DPSCs on graphene oxide-based substrates. Their results indicated an upregulation of odontogenic gene expression with graphene oxide-based substrates, in comparison to the control (glass-based substrates). The same group later observed higher osteogenic rather than odontoblastic differentiation (Xie et al., 2017). However, in vitro studies do not provide robust data distinction between osteogenic and odontogenic differentiation, and further in vivo studies are required to make this distinction.

More recently, graphene-based materials have gained significant attention within the dental field, including graphene coated implants (Jung et al., 2015; Ren et al., 2017), incorporation into commercial dental materials such as adhesives and resins (Bregnocchi et al., 2017; Sun et al., 2018), and as

Table 1f. In vitro and ex vivo studies of alternative antimicrobial strategies highlighting antimicrobial type, origin, study design, usage, study and control groups, assessment method and duration, microorganisms tested and main findings.

<table>
<thead>
<tr>
<th>Alternative antimicrobial/origin</th>
<th>Study design/usage</th>
<th>Study groups</th>
<th>Control groups</th>
<th>Microorganism</th>
<th>Assessment method/duration</th>
<th>Main findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AgNPs in an aqueous vehicle/Khemia, São Paulo, Brazil</td>
<td>Bovine dentine blocks</td>
<td>Irrigant</td>
<td>94 ppm AgNPs solution 2.5 % NaOCl 2 % CHX</td>
<td>Saline without inoculum</td>
<td>E. faecalis biofilm and infected dentinal tubules</td>
<td>LIVE/ DEAD staining</td>
<td>AgNPs showed variable antimicrobial efficacy; however, this was inferior to NaOCl</td>
</tr>
<tr>
<td>Bioactive glass S53P4 Medicament</td>
<td>Contra-lateral human premolar teeth</td>
<td>Bioactive glass Ca(OH)₂</td>
<td>No medicament</td>
<td>E. faecalis</td>
<td>Sampling of dentine chips/ SEM 10 d</td>
<td>Bioactive glass, in its current format, was less efficient than standard Ca(OH)₂</td>
<td>Zehnder et al., 2006</td>
</tr>
<tr>
<td>Bioactive glass S53P4 Medicament</td>
<td>Human dentine block model</td>
<td>Bioactive glass Ca(OH)₂</td>
<td>2 % CHX gel 2 % metronidazole gel</td>
<td>Saline</td>
<td>E. faecalis</td>
<td>CFUs/ inhibition of growth 1, 3 and 5 d</td>
<td>Bioactive glass S53P4 antimicrobial efficacy was superior to Ca(OH)₂, but inferior to metronidazole and CHX gel</td>
</tr>
<tr>
<td>Bioactive glass S53P4</td>
<td>Human root model</td>
<td>Bioactive glass Ca(OH)₂</td>
<td>Ca(OH)₂ 1 % CHX bioactive glass Ca(OH)₂ plus point Activ point</td>
<td>Saline</td>
<td>E. faecalis and S. mutans</td>
<td>CFUs 7 d</td>
<td>CHX-impregnated medicaments were more efficient than alkaline-pH-acting medicaments</td>
</tr>
<tr>
<td>TAMP bioactive glass Medicament</td>
<td>Human dentine block model</td>
<td>TAMP-bioactive glass 100 mg/mL-DAP 1 mg/mL 0.02 % AgNPs gel</td>
<td>Without bacterial contamination</td>
<td>E. faecalis biofilms and infected dentinal tubules</td>
<td>Biofilm disruption assay/ CFUs/SEM 1 and 7 d</td>
<td>All tested medicaments resulted in significant biofilms reduction</td>
<td>Sadek et al., 2019</td>
</tr>
</tbody>
</table>
bioactive cements (Dubey et al., 2017). To enhance root canal disinfection, the synthesis of AgNPs on an aqueous graphene oxide matrix has shown promising preliminary results in terms of antimicrobial activity and biofilm disruption (Ioannidis et al., 2019).

Their versatile and promising properties position graphenes as suitable candidates for investigation within RET. Emerging studies on them as intra-canal disinfectant agents are summarised in Table 1.

**Pectin**

Pectin is a natural plant-specific carboxylated polysaccharide extracted from fruits or vegetables. Pectin provides mechanical strength for the cell walls of plants and are important for various cellular processes, such as water absorption, morphological development, and ripening of fruits (Redondo-Nevado et al., 2001; Vincken et al., 2003). Pectins are known for their gelling, thickening and emulsifying properties, hence their wide use in the food industry. Its emulsification property is affected by the chemical characteristics of the raw material as well as extraction methods used and can be modified using chemical and enzymatic treatments.

The gelling properties of pectin became of interest due to its possible use as an *in situ* biocompatible gelling system for bone tissue engineering (Munarin et al., 2010a; Munarin et al., 2011; Munarin et al., 2012), an injectable cell delivery system (Mishra et al., 2008), and a drug delivery system (Ishii and Matsunaga, 2001; Munarin et al., 2010b). The structure and features of pectins depend on the plant species and tissues. However, some characteristics are common to all of these polysaccharides. Two main structural categories have been recognised, the smooth and hairy (or ramified) regions (Varoni et al., 2012).

Plant-derived pectins have been investigated as candidates for surface nano-coating of orthopaedic and dental titanium implants, due to their ability to enhance osteogenic differentiation of osteoblasts. Folkert et al. (2016) investigated the effect of coating titanium implants, with unmodified pectin and enzymatically modified pectin extracted from potato pulps, *on in vitro* cell proliferation, mineralisation, and osteogenic differentiation of osteoblast-like cell lines and primary mice osteoblasts. The study confirmed that both types of pectin coating enhanced cell proliferation, mineralisation, and osteoblastic differentiation – particularly modified pectins with a high content of galactose (Folkert et al., 2016).

Conversely, Gurzawska et al. (2017) evaluated the effect of nano-coating titanium implants with plant-cell-wall-derived rhamnogalacturonan-I (pectins), on the bone healing and osseointegration of implants in the tibia of a rabbit model. The results showed no significant difference between coated and non-coated implants.

In dentistry, Nguyen et al. (2013) found that different pectins (LM-, HM- and AM-pectin) coated liposomes, adsorbed the hydroxyapatite to the tooth surfaces *in vitro* which suggested their possible usage as a protective coating on tooth enamel. Furthermore,

<table>
<thead>
<tr>
<th>Alternative antimicrobial/origin</th>
<th>Study design/usage</th>
<th>Study groups</th>
<th>Control groups</th>
<th>Microorganism</th>
<th>Assessment method/duration</th>
<th>Main findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(lactic-co-glycolic acid) nanoparticles with methylene blue as a photosensitiser</td>
<td>Human root model Irrigator</td>
<td>Methyleneco blue loaded nanoparticles with/without light</td>
<td>No light/ no methylene blue nanoparticles</td>
<td><em>E. faecalis</em> in planktonic and biofilm forms</td>
<td>CFUs/ SEM Light application for 5 min with a wavelength of 665 nm</td>
<td>Methylene blue loaded nanoparticles reduced <em>E. faecalis</em> counts in both planktonic phase and within root canals</td>
<td>Pagonis et al., 2010</td>
</tr>
<tr>
<td>Curcumin/ indocyanine green as photosensitiser</td>
<td>Well plates Irrigator</td>
<td>5.25 % NaOCl, 0.2 % CHX, 2 % CHX, 40 mmol/L curcumin with LED 1 mg/mL indocyanine green with diode laser</td>
<td>No exposure to irrigation solutions or photosensitisers</td>
<td><em>E. faecalis</em> in planktonic and biofilm forms</td>
<td>CFUs/ crystal violet assay LED wavelength of 450 nm Diode laser wavelength of 810 nm</td>
<td>Photoactivated curcumin antimicrobial efficacy was very close to standard irrigating solutions tested</td>
<td>Pourhajibagher et al., 2018</td>
</tr>
<tr>
<td>rGO-Cur photosensitiser</td>
<td>Human root model Irrigator</td>
<td>rGO-Cur LED group rGO-Cur with LED 2.5 % NaOCl</td>
<td>Only bacterial suspension</td>
<td><em>E. faecalis</em> biofilms</td>
<td>MIC/ SEM/ real-time qPCR Wavelength of 450 ± 30 nm with 300 s</td>
<td>The combined usage of rGO-Cur with LED showed promising results in terms of antimicrobial efficacy and biofilm inhibition</td>
<td>Ghorbanzadeh et al., 2020</td>
</tr>
</tbody>
</table>
in vivo usage of pectin in combination with a chitosan scaffold within RET in dog endodontic lesions was assessed (Palma et al., 2017). However, the mineral content and root closure were better using a blood clot alone, in comparison to a pectin-chitosan scaffold (Palma et al., 2017).

Pectin is reported to have bactericidal effects against the most widely distributed pathogenic and opportunistic microorganisms. It was also found that higher concentrations (> 2 %) had an inactivating effect on a therapeutic bacteriophage and decreased the antimicrobial activity of penicillin (Men’shikov et al., 1997). Cinnamaldehyde pectin extracted from the papaya puree were found to have antimicrobial properties (Arrigoni and De Tullio, 2002; Du et al., 2012), is the ionisable hydroxyl groups (Du et al., 2012), is the light-yellow water-soluble ketolactone with two especially for natural collagen synthesis. This white essential vitamin for the health of body tissues and structural variations, and antimicrobial properties, makes injectable pectin gels an excellent candidate to investigate for regenerative endodontics.

**Ascorbic acid**

Ascorbic acid, commonly known as vitamin C, is an essential vitamin for the health of body tissues and especially for natural collagen synthesis. This white to light-yellow water-soluble ketolactone with two ionisable hydroxyl groups (Du et al., 2012), is the most abundant antioxidant vitamin within plant cells (Smirnoff, 2000).

As an electron-donor, ascorbic acid is known to have several physiological and biochemical properties (Arrigoni and De Tullio, 2002; Du et al., 2012). Ascorbic acid has antimicrobial properties against several bacteria and viruses, ranging from bacteriostatic to bactericidal that was reported to increase by time (Goldschmidt, 1991).

Ascorbic acid also has potent anti-inflammatory properties (Diomede et al., 2020), therefore; positively influencing host-defence mechanism towards tissues repair during infection (Goldschmidt, 1991). Its antiscorbutic properties play a pivotal role in the synthesis of collagen through its ability to act as an electron-donor maintaining the ferrous in collagen hydroxylases in an active state (Du et al., 2012). Ascorbic acid’s potential influence on the formation of dentine collagen matrix is of particular interest to researchers in pulp-dentine complex regeneration (Balic et al., 2009).

Within the dental field, ascorbic acid has been advocated as a biocompatible reducing agent integrated into commercial monomers and composite systems. Indeed, 10 % ascorbic acid was found to restore the dentine bonding strength to adhesive resins after deproteinisation with 5 % NaOCl irrigation solution (da Cunha et al., 2010; Furuse et al., 2014; Morris et al., 2001). Additionally, the integration of ascorbic acid within dental monomer increased DPSCs proliferation rate and decreased unwanted cellular morphology alterations due to the monomer treatment (Diomede et al., 2020).

Furthermore, its potential role in promoting regeneration of the dental pulp, as a novel endodontic compound, has been suggested (Diomede et al., 2020). Its relative cheap cost, ease of use, antioxidant properties, and tissue repair properties makes it an

### Table 2. In vivo human and animal studies of alternatives antimicrobial strategies highlighting antimicrobial type, origin, study design, usage, study and control groups, assessment method and duration and main findings.

<table>
<thead>
<tr>
<th>Material/origin/preparation</th>
<th>Study design/usage</th>
<th>Study groups</th>
<th>Control groups</th>
<th>Assessment method/duration</th>
<th>Main findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EEP/Commercially available</td>
<td>Chronically exposed primary teeth Irritant</td>
<td>3 % NaOCl 12.5 % alcoholic extract of miswak 11 % EEP</td>
<td>0.9 % saline</td>
<td>Paper points sampling/ CFUs Pre- and post-irrigation samples</td>
<td>11 % EEP showed disappointing results with no significance difference from the control</td>
<td>Shingare and Chaugule, 2011</td>
</tr>
<tr>
<td>Egyptian propolis/El Monofia province</td>
<td>Immature non-vital dogs’ teeth with induced periapical infection Medicament for RET</td>
<td>Propolis paste with propolis plug Propolis paste with MTA plug TAP with propolis plug TAP with MTA plug</td>
<td>No medicament No intervention</td>
<td>Antimicrobial efficacy: CFUs/reduction in colony counts 3 weeks Regenerative outcome: radiographic/ histopathologic evaluation 2 weeks, 1 and 2 months</td>
<td>Propolis showed promising results, comparable to TAP, in terms of antimicrobial efficacy and hard and soft tissue deposition</td>
<td>El-Tayeb et al., 2019</td>
</tr>
<tr>
<td>Propolis/Farmácia de Manipulação Nova Derme</td>
<td>Immature dog teeth with induced periapical infection Medicament for RET</td>
<td>TAP 1 % propolis paste</td>
<td>No medicament No intervention</td>
<td>Histological analysis 7 months</td>
<td>Propolis showed promising results, superior to TAP, in terms of hard and soft tissue deposition</td>
<td>Pagliarin et al., 2016</td>
</tr>
</tbody>
</table>
excellent candidate to investigate for regenerative endodontics.

Synthetic materials and strategies

Bioactive glass
In the early 90s, Hench and colleagues developed a novel Class A bioactive glass-ceramic material composed of silica and other components such as calcium and phosphate (Hench and Paschall, 1973; Hench et al., 1971). Following this discovery, bioactive glass (calcium sodium phosphosilicate) received considerable clinical interest within the dental field as a versatile material—as a bone substitute for tissue regeneration (Norton and Wilson, 2002; Pereira et al., 2018), an implant coating material (Xuereb et al., 2015), a drug delivery material (Wu and Chang, 2014), and an additional component within various restorative materials (Sauro et al., 2012; Tirapelli et al., 2011). This attracted research attention was largely related to its inherent material advantages such as antimicrobial activity (Munukka et al., 2008; Waltimo et al., 2007; Zhang et al., 2010), biocompatibility, and hard tissue regenerative potential (El-Gendy et al., 2015; El-Gendy et al., 2013; El Shazley et al., 2016). The mechanism of antimicrobial action of bioactive glass is attributed to different factors—its alkaline pH, the sustained release of silica and/or calcium phosphate ions, and specific glass composition (Gubler et al., 2008; Zhang et al., 2010). However, because conventional micron-sized bioactive glass demonstrated mild to moderate antimicrobial activity, specifically against E. faecalis, material improvements were consistently ongoing (Krithikadatta et al., 2007; Waltimo et al., 2007; Zehnder et al., 2006).

Advances with nano-technology fabrication led to the development of nano-scale bioactive glass (Lei et al., 2010). The nano-scale bioactive glass 45S5 was found to increase the pH in a solution and increase silica release by a factor of 10 in comparison to μm-sized bioactive glass, resulting in improved antimicrobial effectiveness (Waltimo et al., 2007). Increased ion release of nano-scale bioactive glass also enhanced cytocompatibility and tissue regeneration properties (Lei et al., 2010; Wang et al., 2020); nano-scale bioactive glass also promoted dentine mineralisation of higher stability and acid resistance compared to micron-scale glass particles (Sheng et al., 2016).

Various types of bioactive glass have been specifically developed and investigated such as TAMP bioactive glass (Sadek et al., 2019) and mesoporous bioactive glass (Wu et al., 2011; Yan et al., 2004). Furthermore, with improved material science, the ability to customise bioactive glass functionalised structures with the addition of antimicrobial and regenerative agents became possible (Ribeiro et al., 2020). The combined addition of silver ions to mesoporous bioactive glass has shown promising results in terms of improved antimicrobial effectiveness (Gargiulo et al., 2013). More sophisticated hybrid systems have also been developed, such as the incorporation of silver-doped bioactive glass within hydrogels with promising antimicrobial, anti-inflammatory, and DPSCs differentiation potential (Wang et al., 2015; Zhu et al., 2019).

Although at an early stage of research, studies expanding its use as a disinfectant agent for endodontics (Atila-Pektaş et al., 2013; Krithikadatta et al., 2007; Zehnder et al., 2006) and RET (Sadek et al., 2019) have been performed and summarised in Table 1.

PDT
PDT, also known as photodynamic inactivation or photoactivated disinfection, is advocated as an adjunct antimicrobial approach for clinical disinfection of the complex root canal system (Gursoy et al., 2013; Plotino et al., 2019). This specialised technique involves the vibrant interaction between a photosensitising agent (photosensitiser) and a light source (Hamblin and Hasan, 2004; Konopka and Goslinksi, 2007). This interaction leads to the production of reactive oxygen species, such as free radicals and singlet oxygen, resulting in oxidative and cytotoxic damage to the target cells (Hamblin and Hasan, 2004; Konopka and Goslinksi, 2007).

The antimicrobial mechanism of PDT can be explained based on the direct effect on extracellular molecules mediated by singlet oxygen of high chemical reactivity and the indirect photodamage to the polysaccharide bacterial biofilm (Konopka and Goslinksi, 2007; Wainwright and Crossley, 2004). This dual activity is reported as a significant advantage over currently used antibiotics (Konopka and Goslinksi, 2007; Plotino et al., 2019), with effectiveness against antibiotic-sensitive and antibiotic-resistant microorganisms (Wainwright and Crossley, 2004). Furthermore, there is no evidence of bacterial resistance to the various metabolic pathways associated with the action of singlet oxygen or free radicals (Dias et al., 2020; Konopka and Goslinksi, 2007).

The effect of PDT on the surrounding stem cells has been investigated. Li et al. (2020) found that the application of PDT provided an inductive microenvironment for SCAP growth. Quantitative reverse transcriptase-polymerase chain reaction also resulted in a positive expression of platelet-derived growth factor and vascular endothelial growth factor. In-line with the above, PDT resulted in greater viability of apical papilla cells (Deluca et al., 2021) and significantly less cytotoxicity compared to NaOCl irrigation (George and Kishen, 2007; Gomes-Filho et al., 2016).

Within dentistry, the application of PDT is expanding into different areas, such as treatment of head and neck cancer (Grant et al., 1993; Hopper, 2000), oral plaque biofilms (Tahmassebi et al., 2015; Wood et al., 2006), treatment of peri-implantitis
(Bassetti et al., 2014; Bombeccari et al., 2013), and root canal disinfection (Asnaashari et al., 2017; Bonsor et al., 2006; de Miranda and Colombo, 2018; Soukos et al., 2006). More recently, PDT has been recommended as a positive adjunct in RET protocols (Deluca et al., 2021; Devaraj et al., 2016). However, to date, this is scarcely documented in vivo. Successful clinical and radiographic outcomes, in terms of thickening of dentinal walls and apical closure, following the adjunct use of tolonium chloride photosensitiser followed by platelet-rich fibrin (Johns et al., 2014) or collagen resorbable matrix (Abdel Hafiz Abdel Rahim et al., 2019) are reported.

Although most reported studies utilised PDT with aid of an intra-canal optic fibre, Nunes et al. showed no significant difference in bacterial reduction when an intra-canal optic fibre was not used. However, in this in vitro study, all teeth were decoronated and only standard 15 mm root segments were utilised (Nunes et al., 2011). The reduced oxygen concentration within the root canals, especially in deep irregularities, can directly affect the formation of cytotoxic oxygen derivatives and reduce the antimicrobial efficacy (Nunes et al., 2011). Modifying the optical fibre tip to improve the clinical performance has been recommended (George and Walsh, 2011).

Chemical phenothiazine dyes such as methylene blue and toluidine blue (tolonium chloride) are often reported within endodontic protocols (Gursoy et al., 2013; Siddiqui et al., 2013). However, potential adverse effects, such as staining and discoloration are documented (Plotino et al., 2019; Ramalho et al., 2017). To overcome this clinical limitation, attempts such as evaluating a therapeutic efficacy window of the chemical dyes have been tested (Gursoy et al., 2013; Plotino et al., 2019). Obliteration of dentinal tubules as a result of viscous photosensitiser substances impregnating the dentine surface is also reported (Plotino et al., 2019). Clinically, this could reduce the bond strength between the root filling material and dentinal walls (Shahravan et al., 2007).

Therefore, to overcome the above limitations and enhance clinical outcomes, research has recently focused on the development of novel formulations, such as polymer-based nanoparticle photosensitiser (Gil-Tomás et al., 2007; Koo et al., 2007; Shrestha and Kishen, 2014). Nanoparticle-based photosensitisers have several advantages over standard photosensitising molecules such as:

1. increasing production of reactive oxygen species;
2. reducing the possibility of multiple-drug resistance;
3. providing selective treatment by localised delivery agents;
4. having a nonimmunogenic nature of nanoparticle matrix (Koo et al., 2007; Pagonis et al., 2010).

Examples of novel photosensitiser suggested for intra-canal disinfection include poly(lactic/glycolic) acid nanoparticles loaded with methylene blue (Pagonis et al., 2010). The use of curcumin solution as a photosensitiser has gained significant scientific interest (da Frota et al., 2015; Ghorbanzadeh et al., 2020; Neelakantan et al., 2015; Pourhajibagher et al., 2018; Sotomil et al., 2019). Chitosan nanoparticles functionalised with rose-bengal photosensitiser were also found to stabilise the structural integrity of root dentine in vitro by photo-crosslinking the collagen, resulting in sufficient elimination of biofilms, the stabilisation of the dentinal matrix (Shrestha and Kishen, 2014), and significant inactivation of bacterial endotoxin lipopolysaccharides (Shrestha et al., 2015). Emerging studies are currently being published of a novel photosensitiser as an adjunct for intra-canal disinfection, with promising results, as summarised in Table 1.

Concluding remarks and future perspectives

Adequate disinfection of the root canal system during RET is a prerequisite for successful regeneration of the pulp-dentinal complex. However, it should be achieved while maintaining a conducive environment for stem cell survival and proliferation. Although currently adopted antimicrobial protocols provide acceptable disinfection, the clinical outcomes are still unpredictable and far from ideal. Key limitations, such as coronal discolouration, cell cytotoxicity, difficulty of removal from the root canal, development of sensitisation and resistant bacterial strains are widely documented within the literature.

There is a growing interest in the exploration of alternative antimicrobial strategies within RET for a predictable biological outcome. Despite the above-mentioned promising results of various new strategies, it is noteworthy that currently available data are mostly drawn from in vitro and limited animal studies using single bacterial species, mainly E. faecalis. Further investigations into the effect of the proposed antimicrobials against complex polymicrobial biofilms involved in endodontic infections is of extreme importance to finalise the conclusion concerning the use of these materials in RET. Therefore, the development and testing of the proposed alternative antimicrobial materials within a well-controlled in vitro, followed by in vivo, studies are required.

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Web reference


Editor's note: There were no questions from reviewers for this paper, therefore there is no Discussion with Reviewers section. The Scientific Editor responsible for this paper was Thimios Mitsiadis.