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A review of common intracanal medicaments in endodontic regeneration treatments

Maryam Babaahmadi

Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran Iran

ABSTRACT

Purpose: Nowadays, regenerative endodontics treatment is recommended in the case of loss of vitality of the dental pulp and the presence of a suitable dental structure. One of the primary reasons for failure after regenerative endodontics treatments is the remaining of bacteria in the root canal. Thus, to improve the prognosis of root canal treatment, it is recommended to use different intracanal medicaments including antibiotics and calcium hydroxide as complementary agents. This study aimed to review common intracanal medicaments in the treatment of endodontic regeneration.

Materials and Methods: In this review study, the keywords of regenerative endodontics, pulp revascularization, paste antibiotic, intracanal medicament, and calcium hydroxide were searched in the Google, Google Scholar, PubMed, SID, ISI Web of Science, and IranMedex databases from 2018 to 2023. Articles that met the inclusion criteria were included in this study.

Results: 39 studies were included in this study. Common intracanal medicaments (triple, double antibiotic pastes, calcium hydroxide, etc.) that are commonly used in regenerative endodontic treatment were used. Both the concentration and duration of exposure to the medicaments affect the viability of stem cells. TAP (Triple Antibiotic Paste) has the highest antibacterial effect against E. faecalis and the highest rate of discoloration. Calcium hydroxide as a medicament will probably have a positive impact on the release of growth factors.

Conclusion: It is better to use antibiotic medicaments with a lower concentration at times between 24 hours and 48 hours for dental root canal disinfection so it does not interfere with the viability of stem cells.

Keywords: regenerative endodontics, intracanal medicaments, triple antibiotic paste, calcium hydroxide

Address for correspondence:

Maryam Babaahmadi,

Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran Iran

E-mail: maryamba472@gmail.com

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INTRODUCTION

Treatment of the roots of permanently damaged immature necrotic teeth is a clinical challenge. Among different treatment protocols, RE (Regenerative Endodontic), defined as "biologically based" treatments, provides better results in terms of tooth growth and viability [1]. In this method, the differentiation potential of stem cells is used to reconstruct the dental structure [2, 3]. In pulp revascularization treatment, which is a subset of regenerative endodontics treatments, there are three primary components: disinfection of the root canal, bleeding in the root canal for recruitment of stem cells, and finally coronal barrier placement and tooth restoration [4-6]. The goal of RE treatments is to achieve biological peri-apical wound healing, increase the width and length of the root, and possibly restore the function of the dentin-root complex and periapical tissues, which disappear due to trauma or infection [7, 8].

Optimal disinfection of the root canal system is necessary to achieve the desired results in REs. It is necessary to remove the microorganisms in the root canal system [9]. Due to the thinness of the root canal walls and their fragile nature, conventional instrumentation of immature permanent teeth with pulp necrosis cannot be performed [10]. Also, in immature permanent teeth, the diameter of the apex mostly exceeds the diameter of the largest files and challenges mechanical instrumentation. Additionally, mechanical preparation may weaken the fragile and thin walls of the roots. Thus, root canal instrumentation with irrigation solutions is recommended [11, 12]. RE is more dependent on intracanal medicaments with antimicrobial activity to provide a sterile environment for pulp reconstruction [13].

Previous studies have indicated that the successful treatment of the pulp dentin complex occurs only when the canal space is free of bacteria [9, 14]. However, due to the complex anatomy of the root canal system in different teeth, chemo-mechanical preparation alone fails to infect and clean the canal [15, 16]. Currently, the most common medicaments used in RE are TAP (Triple Antibiotic Paste) with a concentration of 0.1 mg/ml or 0.01 mg/ml, Double Antibiotic Paste (DAP), and Calcium Hydroxide to effectively remove bacteria from the samples [11, 15]. TAP was first used and introduced by Hoshino and consists of ciprofloxacin, metronidazole, and minocycline [17, 18]. This medicament can be used effectively to remove bacteria from the root canal system [16]. In RE, Triple Antibiotic Paste (TAP) was used for the first time in 2004 [19]. It has been shown that

TAP has an optimal antimicrobial effect and creates a suitable environment for binding to stem cells [20].

Additionally, many studies have indicated that the use of low concentrations of TAP (0.1 mg/ml to 1 mg/ml) has the maximum effect against Enterococcus faecalis biofilm and the least side effects on viability, binding, and proliferating the Dental Pulp Stem Cells (DPSC) and Stem Cells of the Apical (SCAP) [17, 21-23]. To prevent tooth discoloration caused by tetracyclines, classic TAP with DAP was modified by replacing minocycline with alternative antibiotics such as amoxicillin, clindamycin, doxycycline, or cephalosporins [24]. Thus, replacing TAP with DAP has been recommended to prevent the effect of minocycline on dentin discoloration. Iwaya et al. for the first reported the use of DAP as an intracanal medicament in a clinical case of RE [25, 26]. This medicament shows direct and significant antibacterial impacts on immature teeth [27]. It has been shown that these antibacterial effects can occur without significant negative effects on the viability, proliferation, and mineralization of DPSCs [27, 28].

Calcium Hydroxide $Ca(OH)_2$ is another intracanal medicament used in Regenerative Endodontic Procedures (REPs). It seems to be less effective than TAP in its antibacterial capacity, but it has several advantages over TAP, such as lack of discoloration, less cytotoxicity for stem cells, more viability, proliferation of stem cells on treated dentin, promoting growth factor release from treated dentin, and easier removal from root canals. Also, $Ca(OH)_2$ in REPs is less likely to reduce fracture resistance due to relatively short-term use as an intracanal dressing for 1 weeks to 4 weeks [11, 29, 30]. However, it has some limitations in removing several microorganisms that are mostly present in cases of persistent root infection such as Enterococcus faecalis [15].

Several studies have been conducted on the effects of different intracanal antiseptic medicaments. The results of some of these studies have revealed that the use of these medicaments in regenerative endodontic treatments may be challenging and thus affect the success of the treatment. For example, it has been reported that the use of medicaments in regenerative endodontics treatments can lead to unfavorable tooth discoloration and a negative impact on the proliferation of tooth stem cells [13, 31-36]. Also, the effects of using these medicaments in regenerative endodontic treatments can have favorable impacts such as the release of growth factors from dentin [37-39]. However, no comprehensive study has been conducted in this field until now. Thus, the present study aims to review the challenges (unfavorable tooth discoloration and negative impacts on the viability of stem cells) and positive impacts (antimicrobial, release of growth factors from dentine) of common antibiotics in endodontic regeneration treatments.

MATERIAL AND METHOD

The databases of IranMedex and SI Web of Science, PubMed, and Google Scholar were searched from 2018 to 2023. The following terms were searched as keywords: Regenerative Endodontics, Pulp Revascularization, Endodontic Medicament, Medicament, Signaling Molecule, Growth Factor, Coronal barrier, calcium hydroxide, Triple Antibiotic Paste (TAP), Double Antibiotic Paste (DAP), and discoloration.

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("Regenerative endodontics") (MeSH) or ("Regenerative endodontic treatment") or ("Regenerative endodontic procedure") or ("Regenerative endodontic protocols") or ("Regenerative endodontic therapy") or ("Endodontic treatment") or ("Pulp revascularization") or ("Pulp tissue regeneration") or ("Pulp regeneration") or ("Pulp biology") or ("Pulp treatment") or ("Root canal revascularization") or ("Root canal treatment") or (Revitalization) or (Regeneration MeSH) or (Endodontics (MeSH) and ("Intracanal medicament") or ("Root canal medicaments") or ("Intracanal dressing") or (Antimicrobials) or ("Antibiotic paste") or ("Double antibiotic paste)" or ("Double antibiotic") or ("Triple antibiotic paste") or ("Triple antibiotic") or (Minocycline (MeSH) or (Metronidazole (MeSH) or (Ciprofloxacin) (MeSH) or ("Calcium hydroxide)"(MeSH) and Discoloration.

Google scholar

Pulp Regeneration OR Regenerative Endodontics and Intracanal medicament and Endodontic Medicament and Medicament and Signaling Molecule and Growth Factor and Coronal barrier and calcium hydroxide and Triple Antibiotic Paste (TAP) and Double Antibiotic Paste (DAP) and Discoloration.

Studies were reviewed based on some inclusion and exclusion criteria. First, the title and abstract of the searched studies were reviewed by two researchers. Selected articles were evaluated in terms of inclusion and exclusion criteria. The references of these articles were also evaluated manually to evaluate the relevant studies. If they met the inclusion criteria, they were included in the study.

Inclusion criteria

In vitro studies regarding the effect of common medicaments in regenerative endodontic treatment on undesirable tooth discoloration, effect on stem cells or the release of growth factors from the dentin wall, and antimicrobial effect.

Studies with full text in English language without restrictions on washing method or intracanal medicaments.

Articles related to the research topic published from 2018 to 2023.

Exclusion criteria

Lack of access the full text of the articles (articles whose only abstract was in English and the full text was in a language other than English).

Duplicate studies and articles searched in different scientific databases.

Systematic, review, animal studies, and conferences.

The following variables were extracted from the studies: Publication name, year, title, authors, and characteristics of the study, results, and conclusions.

RESULTS

A total of 561 studies were obtained through databases introduced through electronic search. After removing duplicates, 245 articles remained. Then, 160 articles were excluded after screening based on the title and abstract. Based on the inclusion and exclusion criteria, 36 articles were excluded by examining the text of the

studies. Finally, 39 studies were included in the study (Figure 1).

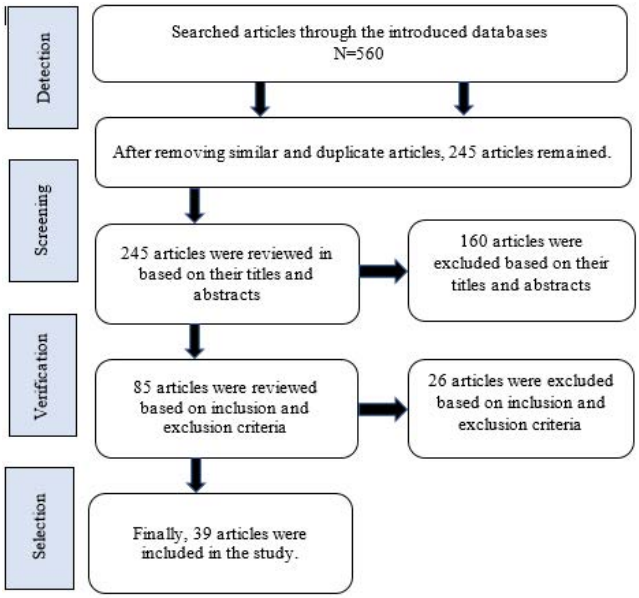

Fig. 1. Study selection steps (study flowchart)

Table 1 presents the effect of medicaments used in regenerative endodontic treatments on the viability of stem cells.

Tab. 1. The effect of medicaments used in regenerative endodontic treatments on the viability of stem cells

Row	Authors and Year	Study Type	Stem Cells	Medicament	Concentration of Medicaments	Results
1	Maru et al (2022) [34]	In-vitro	SHED	calcium hydroxide, doxycycline, potassium iodide, triamcinolone, glutaraldehyde	10 g/mL, 25 g/mL, 50 g/mL and 100 g/mL.	All medicaments significantly reduce cell viability in different concentrations at different times. The highest number of viable cells was observed in SHEDs cultured in calcium hydroxide
2	Bhandi et al (2022) [13]	In-vitro	DPSC	calcium hydroxide, doxycycline, potassium iodide, triamcinolone, and glutaraldehyde, each ranging from 0 (control) to 1000 µg/mL	0 (control), 10 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL, 250 µg/mL, 500 µg/mL, and 1000 µg/mL	All medicaments significantly reduce cell viability at different concentrations at different times. Calcium hydroxide and triamcinolone were beneficial to cell viability at higher concentrations compared to other medicaments at all exposure times.
3	Dencheva et al (2022) [35]	In-vitro	stem cells from apical papilla	Hydrocal Acroseal	4%	The results show that after 22 hours, the groups treated with dental haptens had less viable cells than the control group.
4	Rafatjou et al (2022) [36]	In-vitro	SCAP	Modified Triple Antibiotic Paste (mTAP) TAP	25 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL, 400 µg/ml	The use of mTAP at 100 µg/mg and TAP at a concentration of 200 µg/mg in the first 82 hours, compared to the control group, had no negative toxic impacts on stem cells. Cell viability decreased at higher concentrations, although it was not statistically significant. After 72 hours, the toxicity of concentrations higher than 100 µg/ml of mTAP and 400 µg/ml of TAP significantly reduced the percentage of viable cells.
5	Riaz et al (2022) [8]	In-vitro	DPSC	$Ca(OH)_2$ TAP	$Ca(OH)_2$ of 1 g/ml, 1 mg/ml of TAP	Dentin treatment with TAP for 2 weeks provided a better microenvironment for the viability and attachment of DPSCs compared to Ca (OH)

6	Alfadda et al (2021) [15]	In-vitro	DPSC	Triple Antibiotic Paste (TAP), Calcium Hydroxide ($CaOH_2$), Calcium Hypochlorite ($CaOcl_2$)	$Ca(OH)_2$ (0.75, 1 g/mL); TAP (1 mg/mL)	In dentin surfaces treated with TAP and Ca $(OH)_2$, cell viability increased, but Ca $(OH)_2$ reduced cell viability.
7	Jain et al (2020) [40]	In-vitro	SCAP	TAP Augmentin $Ca(OH)_2$	NR	All three antibiotics significantly reduced the viability of SCAP cells at certain concentrations, while Ca $(OH)_2$ showed a stimulatory effect on SCAP viability.
8	Alzlina et al (2020) [41]	In-vitro	DPSC	DAP DPSC In-vitro $Ca(OH)_2$	NR	Ca $(OH)_2$ significantly increased the viability of stem cells.
9	Gougousis et al (2019) [42]	In-vitro	SHED	TAP $Ca(OH)_2$	NR	In terms of the number of stem cells attached to the dentine surface, Ca $(OH)_2$ showed better results than TAP
10	Sipert et al (2019) [43]	In-vitro	APC	mTAP/Cefaclor mTAP/ $Ca(OH)_2$	250 µg/mL, 62.5 µg/mL, 15.62 µg/mL and 3.9 µg/mL	factor/TAP had the highest level of toxicity for papilla epival cells
11	Pransanti et al (2019) [44]	In-vitro	DPSC	TAP $Ca(OH)_2$ Ledermix	0.1 mg/mL and 1 mg/mL	All three medicaments reduced the viability of stem cells. Among them, Ledermix showed the highest level of toxicity
12	Rahhal et al (2019) [45]	In-vitro	APC	mTAP/Cefaclor $Ca(OH)_2$	250 µg/mL-1000 µg/mL	Ca $(OH)_2$ induced cell proliferation, while mTAP had a negative impact on cell proliferation
13	Gougousis et al (2019) [42]	In-vitro	SHED	TAP $Ca(OH)_2$	NR	Ca $(OH)_2$ showed better results in terms of the number of stem cells on the surface of the root dentin.
14	Khoshkhounejad et al (2019) [1]	In-vitro	APSC	TAP mTAP/ Clindamycin DAP $Ca(OH)_2$ Augmentin	10 mg/ml	TAP had less toxic effect on stem cells than other groups, but Augmentin had the most toxic effect on stem cells.
15	McIntyre et al (2019) [28]	In-vitro	DPSCs	DAP $Ca(OH)_2$	1 mg/mL, 5 mg/mL, and 10 mg/mL	Ca $(OH)_2$ and 1 mg/ml of DAP showed significant negative effects on the proliferation of DPSCs.

Dental Pulp Stem Cells (DPSC), Human Exfoliated Deciduous Teeth Stem Cells (SHED), Stem Cells of the Apical Papilla (SCAP), Apical Papilla Cells (APC), mTAP (Metronidazole, ciprofloxacin, clindamycin), NR: Not Reported.

Table 2 presents the results related to the effect of medicaments dentin.
used in endodontics on the release of growth factors from dental

Tab. 2. The effect of medicaments used in regenerative endodontic treatments on the release of growth factors from dental dentin

Row	Author and Year	Study Type	Tested Groups	Release of Growth Factors
1	Akhila et al (2021) [46]	In-vitro	Group A: (2% chlorhexidine gluconate); Group B: (2.5% sodium hypochlorite); Group C: calcium hydroxide powder ($Ca(OH)_2$); Group D: Triple Antibiotic Paste (TAP) (minocycline 100 mg + ciprofloxacin 200 mg + metronidazole 500 mg); and one control reagent group, i.e., Group E: (normal saline)	Both chlorhexidine Gluconate 2% root canal washing and calcium hydroxide intracanal medicament significantly induced TGF-1β release.
				2.5% sodium hypochlorite had a negative impact on growth factor release.
				TAP showed a neutralizing effect similar to the control group (normal saline).

2	Ferreira et al (2020) [37]	In-vitro	1- Chlorhexidine 2%, 2- NaOCl 2.5%, 3-TAP, 4-calcium hydroxide, and 5- EDTA 4%	Both intracanal medicaments released a small amount of TGF- β1 compared to EDTA (11%) (The lowest amount was related to TAP).
				2% chlorhexidine released TGF- β1 more than 2.5% NaOCl. However, No significant difference was observed between 10% EDTA and 2.5% chlorhexidine
				The amount of TGF- β1 released in 10% EDTA was significantly higher than 2.5% NaOCl.
				VEGF was not released from any group
3	Ferreira et al (2020) [37]	In-vitro	1-TAP (7days) then calcium hydroxide 2% and EDTA 10% 2-calcium hydroxide (7 days) then Chlorhexidine and EDTA 10% 3- EDTA 10%	There was no significant difference between 10% EDTA and the first group.
				The release of TGF- β1 in the second group was significantly lower than 10% EDTA
4	Cameron et al (2019) [38]	In-vitro	1-TAP 2- Ca $(OH)_2$	Sterile dentin fragments treated with different concentrations of TAP and Ca $(OH)_2$ caused the release of higher amounts of growth factor TGF-ß 1 compared to fragments infected with bacterial biofilm.

Vascular Endothelial Growth Factor (VEGF), Stem Cell Factor (SCF), Insulin-like Growth Factor – I and II (IGF-I and IGF-II), Insulin-like Growth Factor-Binding Protein 1 (IGFBP-1), Macrophage Colony-Stimulating Factor (M-CSF), Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF), Nerve Growth Factor (NGF), Glial Cell-Derived Neurotrophic Factor (GDNF), Epidermal Growth Factor (EGF).

Table 3 presents the results related to the effect of medicaments used in endodontics on tooth discoloration in the studies.

Tab. 3. The effect of medicaments used in regenerative endodontics on tooth discoloration

Row	Author and Year	Study Type	Medicament Type	Results
1	Betancourt et al (2021) [31]	In-vitro	Ca$(OH)_2$ group, TAP with Doxycycline (DOX) TAP with Clindamycin (CLIN)	TAP with DOX caused the highest crown discoloration among the tested medicament. In contrast, Ca $(OH)_2$ and TAP with CLIN did not cause discoloration in the crown after 28 days.
2	Parikh et al (2021) [32]	In-vitro	calcium hydroxide Ca$(OH)_2$, calcium hydroxide with chlorhexidine Ca$(OH)_2$ with CHX), Triple Antibiotic Paste (TAP), Silver Diamine Fluoride (SDF)	The highest discoloration was observed in the TAP group followed by the SDF group, while Ca$(OH)_2$ and Ca$(OH)_2$ with CHX groups did not cause significant discoloration in the crown)
3	Sabrah et al (2020) [33]	In-vitro	TAP DAP MTAP (contains diluted minocycline) and methyl cellulose MDAP (containing methyl cellulose)	TAP and MTAP significantly caused the highest rate of discoloration.
4	Venkataraman et al (2019) [47]	In-vitro	TAP mTAP/ amoxyclav mTAP/ clindamycin	TAP containing Minocycline or amoxyclav caused more discoloration compared to TAP modified with clindamycin, while the rate of discoloration in TAP group containing clindamycin was not significant.
5	Shokouhinejad et al (2018) [48]	In-vitro	TAP	TAP containing minocycline causes tooth discoloration. Although covering the walls of the pulp chamber with materials that bind to the dentin reduces the rate of discoloration, it does not prevent from its formation.
6	Jagdale et al (2018) [49]	In-vitro	TAP mTAP/cefaclor	Both medicaments caused discoloration in teeth, although TAP caused more discoloration. In both groups, with the increase in the thickness of the temporary restorative material, the rate of discoloration was reduced
7	Afkhami et al (2018) [50]	In-vitro	TAP Ca$(OH)_2$ Ca$(OH)_2$/CHX	TAP, especially in long-term use (3 months), significantly caused more teeth discoloration.
8	AlSaeed et al (2018) [51]	In-vitro	TAP Augmentin Tigecycline	All three of these medicaments at a concentration of 1 ml/mg, when used in hydrogel, compared to using them alone or in a lower concentration, significantly reduced the growth of bacteria, and the discoloration was minimal.

Table 4 shows the effect of antimicrobial medicaments used on the reduction of bacterial load in regenerative endodontics.

Tab. 4. The effect of antimicrobial medicaments used on the reduction of bacterial load in regenerative endodontics

Row	Author and year	Medicament type	Study type	Results
1	Govindaraju et al (2021) [52]	1:TAP, 2: Calcium hydroxide paste; 3: Odontopaste; 4: Sterile saline (0.9%)	In-vitro	The highest inhibitory effect was related to TAP. Odontopaste and calcium hydroxide had a similar effect on E. faecalis.
2	Alfadda et al (2021) [15]	Triple Antibiotic Paste (TAP), Calcium Hydroxide ($CaOH_2$), Calcium Hypochlorite $CaOCl_2$	In-vitro	All medicaments reduced the initial bacterial load. The highest bacterial reduction in the main canal and dentinal tubules was observed in Ca $(OC)_2$ group.
3	Khoshkhounejad et al (2021) [53]	Triple Antibiotic Paste (TAP), Double Antibiotic Paste (DAP), Modified Triple Antibiotic Paste (MTAP)-1, MTAP2, co-amoxiclav, and Calcium Hydroxide (CH)	In-vitro	TAP, DAP, co-amoxiclav, and CH (in its MBC value) could significantly remove E. faecalis biofilm.
4	Zancan et al (2019) [54]	TAP DAP DAP/ $Ca(OH)_2$ $Ca(OH)_2$	In-vitro	TAP and DAP showed the highest reduction in bacterial population. Adding $Ca(OH)_2$ to DAP significantly reduced its antibacterial properties.
5	McIntyre et al (2019) [28]	DAP $Ca(OH)_2$	In-vitro	$Ca(OH)_2$ and 1 mg/ml of DAP showed direct antibacterial effect without significant negative effects on the proliferation of DPSCs.
6	Dall et al (2021) [55]	TAP DAP $Ca(OH)_2$	In-vivo	DAP significantly decreased the number of bacteria compared to the control group $Ca(OH)_2$ and the results were almost similar to TAP.
7	Hussein et al (2019) [56]	DAP $Ca(OH)_2$ Chitosan Nanoparticle	In-vitro	After 12 days, all three medicaments were successful in completely removing the bacterial biofilm. No significant statistical difference was observed between the three medicaments.
8	Jenks et al. 2019 [57]	DAP $Ca(OH)_2$	In-vitro	The use of DAP in concentrations higher than 1 ml/mg for four weeks compared to one week had a significantly better residual anti-biofilm effect. This medicament at a concentration of 1 ml/mg and $Ca(OH)_2$ did not cause residual anti-biofilm effect at any time.
9	El-Tayeb et al. 2019 [58]	TAP at different concentrations	In-vivo	TAP in very low concentrations also significantly reduced the number of bacteria.
10	AlSaeed et al., 2018 [15]	TAP Augmentin Tigecycline	In-vitro	All three of these medicaments at a concentration of 1 ml/mg, when used in hydrogel, significantly reduced the growth of bacteria compared to using them alone or at a lower concentration.
11	Zargar et al. 2018 [59]	TAP at two concentrations Clindamycin $Ca(OH)_2$	In-vitro	TAP and clindamycin groups had significantly more antibacterial effect than $Ca(OH)_2$. No significant statistical difference was observed between them.
12	Gabraei et al. 2018 [60]	TAP $Ca(OH)_2$/CHX	In-vitro	$Ca(OH)_2$/CHX could completely remove bacteria in three days, while TAP needed 7 days to completely remove bacteria.

DISCUSSION

The effect of medicaments used in regenerative endodontic treatments on the viability of stem cells

Most of the studied studies have reported that the use of medicaments reduces the viability of stem cells [13, 34, 61]. Maru et al. (2022) reported that in SHED cells, 10 mL/g, 25 mL/g, 50 mL/g, and 100 mL/g of Calcium Hydroxide, Potassium Iodide, Triamcinolone, And Glutaraldehyde significantly reduced cell viability at different concentrations at different times. The lowest level of toxicity was related to calcium hydroxide [34]. Bhandi et al. (2022) also found that in DPSC cells, concentrations of 0, 25µg/mg, 10µg/mg, 50µg/mg, 100 µg/mg, 250 µg/mg, 500 µg/mg, and 1000µg/mg significantly increased cell viability at different concentrations decreased at different times, and the lowest level of toxicity was related to calcium hydroxide and triamcinolone [13].

Rafatjou et al. (2022) found that the use of mTAP at a concentration of 100 µg/mg and TAP at a concentration of 200 µg/mg in the first 24 hours, compared to the control group, had no negative toxic effects on SCAP. Cell viability also decreased at higher concentrations [36]. In the study by Khoshkhounejad et al. (2019), TAP was identified as the safest medicament for SCAPs, while Augmentin showed some harmful effects. In this study, antibiotic

compounds TAP, DAP, and mTAP were used with a dose of 10 mg/ml, which is the Minimum Inhibitory Concentration (MIC). It was also shown that Augmentin has harmful effects on APSC. They reported that this medicament has destructive effects on SCAP even in low concentrations, which indicates that the cytotoxicity of some chemicals is more related to their intrinsic properties than to their concentration [1].

The study by McIntyre showed that DAP in all concentrations (1 mg/ml, 5 mg/ml, and 10 mg/ml) has specific antibacterial effects, but only the concentration of 1 mg/ml has a significant direct antibacterial effect without leaving significant negative effects on the proliferation of DPSCs. Also, unlike higher concentrations, DAP at a concentration of 1 mg/ml did not negatively affect the alkaline phosphatase activity of cells and their mineralization rate [12, 62]. The AAE recommendation is to use calcium hydroxide or a low concentration of triple antibiotic paste (0.1 mg/ml-1 mg/ml) as an intracanal medicament before REP [63]. It has also been reported that a low concentration of calcium hydroxide (0.01 mg/ml) and TAP (0.01 mg/ml) led to a significant increase in live stem cells compared to the higher concentration on days 1, 3, and 5 [64]. The concentration of 25 g/ml on stem cells is tolerated by the stem cells. Calcium hydroxide is an antiseptic that is approved for intracanal use during endodontic regeneration. Calcium Hydroxide increases the proliferation, osteogenic differentiation, and mineralization of Dental Pulp Stem Cells (DPSCs) through the mitogen-activated protein kinase pathway. This can justify its possible non-toxicity up to a concentration of 25 g/ml [13-34]. It seems that the toxicity of medicaments depends on their concentration. This means that medicaments in lower concentrations have a less toxic effect on stem cells and the cytotoxic effect of medicaments is dependent on their dose. Based on studies, the use of medicaments is not necessarily associated with a negative effect on stem cells. For example, some studies have referred to the non-toxic nature of calcium hydroxide and its capability to induce mineralization of cells [28, 65, 66]. Generally, it seems that some medicaments show acceptable antibacterial efficiency in higher concentrations, which may have a destructive effect on the viability and function of stem cells [1].

The effect of medicaments used in regenerative endodontic treatments on the release of growth factors from dental dentin

Growth factors are biologically active molecules, proteins, or chemical polypeptides that change the cellular response through intercellular communication. The action of various factors that cause dentin demineralization can release these molecules from their resting state in the components of the extracellular matrix [46]. Selecting the appropriate concentration of detergents and medicaments is crucial since it may affect the strength of growth factors and the viability of stem cells, both of which are necessary for pulp-dentin regeneration [39]. Due to the high importance of growth factors on the success of regenerative endodontic treatments, the medicaments used to clean the root canal during regenerative endodontic treatment should not negatively affect the release of these factors as much as possible [37]. There are various groups of growth factors in the dentin matrix, including Bone Morphogenetic Protein (BMP), fibroblast growth factor 2, Transforming Growth Factor β-1 (TGF-β-1), Vascular Endothelial Growth Factor (VEGF), and placenta growth factor, and platelet-derived growth factor [46].

The results of a study by Akhila et al. (2021) showed that calcium hydroxide has an increasing effect on TGF-1β release, while TAP showed a neutral effect similar to the control group (normal saline) [46]. The results of a study by Ferreira et al. (2020) showed that calcium hydroxide is more capable of releasing TGF-ß1 from the dentin of the cervical region of the tooth. They related the release of growth factor amounts after the application of TAP to the low pH of this paste and the presence of minocycline in it [37]. It is a chelating agent. TAP also has a very strong acid property (pH=2.9), which is capable of demineralizing the surface of dentin [10]. In the study by Ferreira et al, the growth factor release rate after using TAP followed by chlorhexidine was 8% much more than when TAP was used alone [37].

One of the reasons for this could be that following the use of chlorhexidine, more amounts of antibiotic paste are removed from the root canal, and thus, the access of EDTA detergent (which is used afterward) to the tooth dentin for the release of the root factor will increase. They concluded that in case of using 2% chlorhexidine as a detergent, calcium hydroxide as a medicament, and EDTA 10% as a final detergent, or using TAP as a medicament and then washing with 2% chlorhexidine, and finally using EDTA 10% as the final detergent, the highest amount of TGFß1 release from the dentin of the cervical region of the tooth will be obtained [37]. In this study, VEGF was not detected after the use of medicaments. As stated before, they reported that the ELISA test used in this study has a detection limit of at least 15.6 pg/ml. This growth factor was less than the detection limit, so it was not detected. Also, in this study, dentin from the cervical region of the tooth was used as it has a lower amount of VEGF compared to the more apical parts [37]. In their study, Cameron et al. (2019) referred to the destructive effects of the remaining bacterial biofilm on TGFß1 release. This means that in the case of using TAP and calcium hydroxide, more amounts of growth factor were released on sterile dentin than when these medicaments were used on dentin infected with bacterial biofilm [38].

The effect of common medicaments used in regenerative endodontics on tooth discoloration

Crown discoloration in teeth in REP procedures has been reported at 40% to 62% [31, 67]. Since REPs are the selected treatment in immature permanent teeth with pulp necrosis, this percentage cannot be ignored. The reason for the discoloration reported after REPs could be due to pulp necrosis, infection of the dentinal tubule with blood after blood clot formation, MTA used as part of the protocol, and TAP used as an intracanal medicament [31]. Using TAP caused discoloration in most studies [1, 31, 32, 49, 50]. This is due to the high concentration of minocycline in TAP used in clinical work, which generally has a concentration between 600 ml and 1000 ml. The binding of minocycline to calcium ions through chelation and the formation of an insoluble precipitate is considered to be the mechanism by which minocycline has a significant tendency to bind to collagen at very low concentration of TAP (pH=2.8), the dentin is demineralized, and the collagen in the root dentin is exposed. The minocycline bound to the collagen is oxidized by exposure to air or bacterial activity, leading to the destruction of the benzene ring. This leads to the formation of the black insoluble quinone compound, which is responsible for the black discoloration of the root dentin [22]. Betancourt et

al. (2021) reported that TAP with doxycycline caused the most crown discoloration among the tested medicaments. In contrast, calcium hydroxide and TAP with clindamycin did not cause discoloration in the crown after 28 days [21]. Parikh et al. (2021) also found that the most discoloration was observed in the TAP group, while calcium hydroxide did not cause a significant discoloration in the crown [32].

Sabrah et al. (2020) showed a significant difference between the rates of discoloration created after using TAP and diluted TAP. This indicates that the final concentration of minocycline in the mixture significantly affects the amount of discoloration. Dentin is effective because its higher concentrations lead to greater bonding with dentin, which is unlikely to be removed after root canal cleaning. However, they also stated that tooth discoloration can be seen even at low doses of minocycline. Sabra et al. stated that medicaments containing minocycline should not be used in tooth root reconstruction, especially when beauty is one of the primary factors in the treatment plan. More studies should be conducted on finding alternative antibiotics to disinfect the root canal without jeopardizing the teeth' discoloration in the facial area [33]. The study by Jagdale et al. (2018) reported that the duration of using TAP was also effective in the discoloration rate and the discoloration increased steadily from the first week to the sixth week [46]. These results were in line with the results of the study by Afkhami et al. (2019) who showed that the discoloration rate after using TAP increased over time. Therefore, they recommended that the use of TAP should be limited to shorter periods [50].

The results of a study by Sabrah et al. (2020) also indicated that although the discoloration rate after using DAP is less compared to TAP, the discoloration is not very favorable in terms of treatment success [33]. Venkataraman et al (2019) also showed that TAP and TAP modified with amoxiclav caused more discoloration compared to TAP modified with clindamycin. The discoloration in the TAP group containing clindamycin after three weeks was not clinically significant. They reported discoloration after the use of TAP containing the antibiotic Amoxiclav due to the formation of chromogenic deposits [47]. Afkhami et al. (2019) showed that TAP compared to calcium hydroxide and calcium hydroxide compounds treated with chlorhexidine significantly caused more discoloration [50]. Although the discoloration caused by medicaments is partially removed after the use of bleaching materials, dentists should be careful in selecting its concentration and the duration of medicament when choosing the type of medicament due to the impossibility of removing it completely [68, 69]. The use of dentin bonding agents to cover the dentin tubules to prevent the medicament from entering the tubules, and the use of different delivery systems to place the medicaments inside the root canal without contact with the pulp chamber of the crown are also recommended to prevent tooth discoloration [1]. According to the studies, only the medicaments used in regenerative endodontic treatments are not the cause of tooth discoloration [70]. It has been shown that blood clots or other scaffolds used in this method or coronal sealing biomaterials such as MTA can also play a role in the treatment discoloration [31, 70, 71]. As a result, the type of scaffold used and also the type of material covering the coronal part of the tooth, in addition to the medicament used, should be considered when evaluating tooth discoloration [70].

Antimicrobial effect of common medicaments used in regenerative endodontics

Biofilm formation is one of the prominent features of root pathogens. In other words, apical periodontitis occurs due to biofilm formation. During REPs, the removal of intracanal bacteria primarily relies on chemical disinfection due to the thinness of the dentin walls and their mechanical limitations. On the contrary, in conventional root canal treatment, intracanal debridement is performed chemo-mechanically, which can be the reason for reporting a higher rate of success in removing bacteria during routine RCTs compared to REPs [72]. Higher concentrations of antibacterial agents used in REPs have caused concern about stem cell toxicity in recent years [53]. Also, insufficient disinfection of the root canal system can lead to direct toxicity of stem cells. Studies have indicated that bacterial DNA, lipopolysaccharide, and lipoteichoic acid can activate innate immune reactions, leading to the destruction of stem cells [73, 74]. Thus, determining and using the minimum concentrations of medicament preparations that can be a safe area for stem cells and effective in removing bacteria from the root canal system cannot be ignored in successful REPs [53]. The results of studies showed that TAP has a high disinfection capacity both in the root canal and at a depth of 100 μm and 200 μm of dentinal tubules, unlike calcium hydroxide, which showed weak antimicrobial activity at both depths [10, 12]. The higher penetration capacity and efficiency of TAP in the depth of dentinal tubules may be due to the lower surface tension of TAP compared to CH. Also, TAP has a low pH and chelating capacity (due to the presence of minocycline in its composition), which significantly increases its demineralization potential [10, 71].

In the study by Zancan et al. (2019), TAP and DAP were more effective than calcium hydroxide and caused the greatest reduction in the bacterial population over 7 days [75]. They stated that a period of 7 days is enough to create the maximum antibacterial activity, and we should reuse the paste in the tooth canal due to the high solubility of these pastes if we want to use these medicaments for a longer period in the root canal [54]. These researchers also found that adding calcium hydroxide to DAP significantly reduced its antibacterial properties. They attributed its reason to the alkaline environment provided by calcium hydroxide and thus its negative effect on the permeability of the bacterial cell wall to the antibiotic. They recommended the combination of antibiotic medicaments with calcium hydroxide should be avoided [76]. In the study by McIntyre (2019), the concentration of 1 mg/ml of DAP produced a significant direct antibacterial effect without causing significant negative effects on the proliferation of DPSCs. The study by Pereira et al. (2019) also reported similar results [76]. Due to the discoloration caused by TAP due to the presence of minocycline, researchers proposed the use of other antibiotics to disinfect the root canal.

Zargar et al. (2018) showed that TAP groups at concentrations of 20 mg/ml and 1000 mg/ml, and clindamycin, which is a broad-spectrum antibiotic, had a significantly greater antibacterial effect than calcium hydroxide. However, no significant statistical difference was observed among these three groups. They concluded that clindamycin at a concentration of 20 mg/ml and TAP with a concentration of 20 mg/ml have the same antibacterial efficacy as TAP with a concentration of 1000 mg/ml and they can remove the 3-week biofilm at a depth of 400 μl from the inner surface of the root canal. As a result, there is no need to use higher concentrations of antibiotic medicaments. In this study, calcium hydroxide not only had a lower antibacterial effect but also its effects at

a depth of 200 µl were limited [59]. AlSaeed et al. (2018) investigated the antibacterial effects of three medicaments TAP, Augmentin, and Tygecycline in different concentrations and with or without the use of hydrogel and evaluated the discoloration rate created after using these medicaments. The results of this study revealed that all three of these medicaments at a concentration of 1 mg/ml, when used in the hydrogel, compared to using them alone (without hydrogel) or at a lower concentration, significantly caused a decrease in bacteria growth and the discoloration was at a minimum level.

Thus, the hydrogel containing TAP with a concentration of 1 mg/ml, Augmentin (a combination of amoxicillin and clavulanic acid), and Tygecycline (a new antibiotic from the tetracycline category) can be used to disinfect canals. Based on the results, they reported the use of these medicaments at a lower concentration (0.1 mg/ml) has no clinical indication [51]. There is no agreement regarding the antibacterial efficiency of calcium hydroxide medicament [59, 76]. For example, Hussein et al. (2019) concluded that both DAP and calcium hydroxide can remove bacterial biofilm after 14 days [56]. These results are in line with the study by Gabriel et al., who showed that calcium hydroxide can remove bacterial biofilm completely [60]. In the study by McIntyre et al. (2019), both calcium hydroxide and 1 mg/ml concentration of DAP had a significant and direct antibacterial effect on the proliferation of DPSCs without leaving significant negative effects [28]. It is recommended that different concentrations of medicaments used in studies with a large sample size should be investigated to find the lowest required concentration that can induce the highest amount of growth factors from the dental dentin matrix and negatively affect stem cells. Also, newer substances should be investigated.

CONCLUSION

Both the concentration and the duration of exposure to medicaments are effective on the viability of stem cells. It is better to use antibiotic medicaments with a lower concentration at times of 24 hours to 48 hours to disinfect the root canal of the tooth so they do not interfere with the viability of stem cells.

TAP has the highest antibacterial effect against E. faecalis and the highest rate of discoloration. Calcium hydroxide as a medicament will probably have a positive effect on the release of growth factors. However, due to the possibility of harmful effects of the substances used in the regenerative treatment of dental pulp, especially medicaments, on the viability of stem cells, tooth color, its resistance, etc., dentists must carefully select the type of these substances and their appropriate concentration to leave less negative effects.

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