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**Sara Ayoub, Ali Cheayto, Sanaa Bassam, Mehdi Najar, Antoine Berbéri & Mohammad Fayyad-Kazan**


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# The Effects of Intracanal Irrigants and Medicaments on Dental-Derived Stem Cells Fate in Regenerative Endodontics: An update

Sara Ayoub[1] · Ali Cheayto[2] · Sanaa Bassam[2] · Mehdi Najar[3,4] · Antoine Berbéri[5] · Mohammad Fayyad-Kazan[6,7]



**Abstract**
Regenerative endodontics is a biologically based treatment designed for immature permanent teeth with necrotic pulp to replace dentin and root structures, as well as dental pulp cells. This procedure has become a part of novel modality in endodontics therapeutic manner, and it is considered as an alternative to apexification. In the last decade, numerous case reports, which describe this procedure, have been published. This therapeutic approach succeeded due to its lower financial cost and ease of performance. Although the clinical protocol of this procedure is not standardized and the effects of irrigants and medicaments on dental stem cells fate remain somewhat ambiguous, however when successful, it is an improvement of endodontics treatment protocols which leads to continued root development, increased dentinal wall thickness, and apical closure of immature teeth. To ensure a successful regenerative procedure, it is essential to investigate the appropriate disinfection protocols and the use of biocompatible molecules in order to control the release of growth factors and the differentiation of stem cells. This is the first review in the literature to summarize the present knowledge regarding the effect of intracanal irrigants and medicaments on the dental derived stem cells fate in regenerative endodontic procedures.

**Keywords** Regenerative endodontics · Intracanal irrigants · Intracanal medicaments · Dental derived stem cells · Signaling molecules · Growth factors · Therapy

## Introduction

The endodontic treatment of immature permanent teeth exposed to trauma or caries is a complicated procedure, due to incomplete root formation [1]. Conventionally, these teeth are disinfected by cleaning and shaping procedures with endodontic instruments and irrigant solutions [2]; thereafter, canal is treated with apexification procedures using mineral trioxide aggregate (MTA), or calcium hydroxide [3], and then filled with gutta-percha. Consequently, this treatment can lead to "arrested root development" state [4]. In the last decades, the field of endodontics-based treatment protocol of immature permanent

✉ Mohammad Fayyad-Kazan
mfayyadk@gmail.com; m.fayyadk@ul.edu.lb

Sara Ayoub
sara.ayoub.1@ul.edu.lb

Ali Cheayto
ali-ch94@hotmail.com

Sanaa Bassam
s.bassam@ul.edu.lb

Mehdi Najar
mnajar@ulb.ac.be

Antoine Berbéri
aberberi@ul.edu.lb

[1] Department of Prosthodontics, Faculty of Dental Medicine, Lebanese University, Hadath, Beirut, Lebanon

[2] Department of Restorative Dentistry and Endodontics, Faculty of Dental Medicine, Lebanese University, Hadath, Beirut, Lebanon

[3] Genetics and Immune Cell Therapy Unit, Faculty of Sciences, University Mohammed Premier, Oujda, Morocco

[4] Osteoarthritis Research Unit, Department of Medicine, Research Center (CRCHUM), University of Montreal Hospital, University of Montreal, Montreal, QC, Canada

[5] Department of Oral and Maxillofacial Surgery, Faculty of Dental Medicine, Lebanese University, Hadath, Beirut, Lebanon

[6] Laboratory of Cancer biology and Molecular Immunology, Faculty of Sciences-I, Lebanese University, Hadath, Beirut, Lebanon

[7] Department of Natural Sciences, School of Arts and Sciences, Lebanese American University, Beirut, Lebanon

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teeth was shifted toward a new treatment strategy, called Regenerative Endodontic Procedure (REP). This is based on creating a conducive microenvironment for stem cell survival and differentiation [5–7], and lead to continued root development and apical closure [8–10]. This procedure acts by disinfecting the root canal, creating a blood clot to the level of the cemento-enamel junction via the evoked-bleeding step, which generate a revascularization and accumulation of growth factors and undifferentiated stem cells in the canal [5, 11, 12]. Interestingly, it has been reported that 60% of endodontists have performed REPs [13].

The clinical steps for REPs have been reported in many studies [6, 14–16] and include:

1 Chemical disinfection of the root canal walls without instrumentation, followed by application of intra-canal medicament during 2 to 4 weeks.
2 Induction of intra-canal evoked-bleeding, accumulation of stem cells, and creating a three-dimensional scaffold and essential growth factors within the root canal lumen to promote stem cell differentiation.
3 Coronal-tight restoration to prevent reinfection.

Therefore, REPs depend on three main factors: disinfection, stem cells, and scaffold. Moreover, REPs recommend providing maximum disinfection without discussing the effect of disinfection agents on Mesenchymal Stem Cells (MSCs) fate [17]. It is noteworthy that, a large population of the MSCs that are introduced inside the root canal lumen upon scratching the periradicular tissues [11], are derived from the apical papilla (SCAPs) [18]. Nowadays, nine populations of dental-derived MSCs are characterized (Fig. 1). These include: dental pulp stem cells (DPSCs); stem cells from human exfoliated deciduous teeth (SHEDs); immature dental stem cells from primary teeth (IDPSC); periodontal ligament stem cells (PDLSCs); dental follicle progenitor cells (DFPCs); stem cells from the apical papilla (SCAPs); alveolar bone-derived mesenchymal stem cells (ABSMCs); gingival mesenchymal stem cells (GMSCs) [19]; and human periapical cyst-mesenchymal stem cells (hPCy-MSCs) [20].

An ideal irrigant and medicament used in REPs must have a balance between their antimicrobial efficacy, and their ability to create an intracanal microenvironment that favors MSCs proliferation and differentiation [17]. Thus, this review will provide a summarized update on the effect of different accepted types of irrigants and medicaments, used in REPs, on the survival and differentiation of MSCs, mainly dental derived SCs.

## Irrigants

Given that dentinal walls are compromised, fragile, and underdeveloped, mechanical instrumentation is therefore avoided in REPs [6]. On the other hand, chemical debridement by irrigation represents the key factor for a successful endodontic treatment [21]. Nowadays, it is established that a single chemical solution is not sufficient to achieve an appropriate disinfection, thus, combined, concomitant or sequential use of two or more irrigating solutions is required [21]. The most widely used agents for chemical debridement are Sodium hypochlorite (NaOCl), EthyleneDiamineTetraacetic acid (EDTA), and Chlorhexidine digluconate (CHX), as well as other various irrigation solutions [22, 23]. To achieve good REPs, an irrigant should promote attachment, proliferation and differentiation of stem cells [24]. Moreover, the disinfection of residual biofilm from the dentin is critical for the release of growth factors into the root canal lumen, and the success of REPs [25]; therefore, adequate irrigant should have various desirable characteristics involving antimicrobial effects, pulp tissue dissolution capacity, and wettability [26]. Likewise, REPs irrigation protocols should have the potency to improve stem cell survival by direct and indirect mechanisms (Table 1).

### Sodium Hypochlorite (NaOCL)

NaOCL is the most widely used irrigant, due to its potent antibacterial and organic debris dissolving actions [39]. This molecule is known as being highly toxic and irritating [40]. Therefore, it is evident that sodium hypochlorite will be a harmful irrigant with a toxic effect on the human bone marrow mesenchymal stem cells (hBM-MSCs) [41].

In REPs, NaOCL is used by 97% of clinical studies, either alone or in combination with other irrigants [42]. Since there is no standardized treatment protocol, Diogenes et al. reported that 63% of cases were irrigated with 3% NaOCl; 36% of cases were irrigated with high concentration (5–6%) of NaOCL; and only 1% of cases used the lowest concentration (~ 1%) of NaOCL [6]. Alkahtani et al. concluded that the toxicity of NaOCl on BM-MSCs is concentration-dependent [41]. Likewise, identical results were obtained with dental-derived MSCs; 6% of NaOCl exerted a deleterious effect on SCAPs survival and differentiation capacity, whereas a concentration of 1.5% NaOCl had less negative effects; typically this effect is concentration-dependent [18]. Other studies observed that DPSCs did not attach to the dentin surfaces treated by 5.25% [27, 30], and 6% of NaOCl [24]. Moreover, an *in vitro* study demonstrated that NaOCl can deteriorate viability, proliferation and differentiation of dental-derived MSCs, such as DPSCs, PDLSCs, and GMSCs in concentration- and time-dependent manner [34].

### EthyleneDiamineTetraacetic Acid (EDTA)

EDTA is a polyaminocarboxylic acid used in endodontics as chelating agent for dentin. It has been reported that EDTA decalcify dentin by interacting with the calcium ions [43]. In

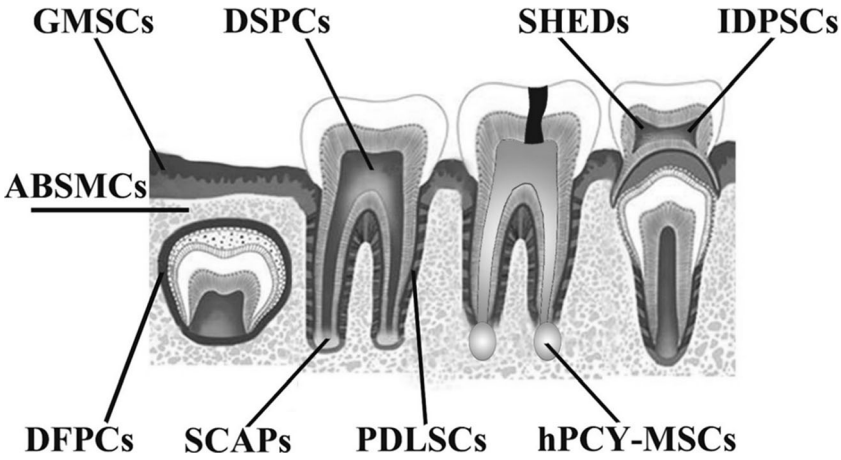


**Fig. 1** Schematic figure illustrating the nine populations of dental-derived MSCs

view of the fact that NaOCl is unable to dissolve the inorganic component of the smear layer, the American Association of Endodontics recommend the use of a concentration of 17% EDTA either as chelating agent for this purpose, or as disinfectant [44, 45]. In 2011, Trevino et al. suggested that the irrigation of root canal dentine with 17% EDTA could promote SCAPs survival and attachment to the root canal dentinal walls [28]. Moreover, Galler et al. demonstrated that dentin conditioning by EDTA stimulate the attachment of DPSCs to the dentin and promote their migration and differentiation into odontoblasts-like cells [31]. Pang et al. showed that EDTA stimulated DPSCs attachment and odontoblastic/osteoblastic differentiation on the EDTA-treated dentin surfaces [29]. Furthermore, it has been indicated that growth factors play a critical role in stem cells migration and differentiation [46]. Since the dentin is an ideal reservoir of signaling molecules [47, 48], EDTA conditioning enable the release of growth factors [such as Transforming Growth Factor beta 1 (TGF-β1), Fibroblast Growth Factor 2 (FGF2), and Vascular Endothelial Growth Factor (VEGF)] dentin tubules [49]. 12% EDTA have been reported to induce dental pulp cells migration by increasing the TGF-β1 expression on these cells [50]. Additionally, this increased expression of growth factors is related to an increased expression of some cytokines, which reveal the immune-inflammatory response occurring during REPs [51]. These studies confirm that EDTA-mediated dentin conditioning is critical for REPs success via demineralization of the dentin and removal of smear layer, which in turn induce growth factors release, and promote stem cells survival, attachment to dentin, and differentiation.

## Chlorhexidine Gluconate (CHX)

CHX is a disinfectant and antiseptic that is used in regenerative endodontics as irrigant and intracanal medication, either alone or in combination with NaOCl, due to its effectiveness against *Candida albicans* and *Enterococcus faecalis* [52].

Widbiller et al. evaluated the direct effect of CHX on SCAPs *in vitro*, and found that a concentration between 2% and $10^{-3}$ % highly affected the viability of SCAPs in a concentration-dependent manner. Conversely, lower concentrations ($10^{-6}$% and $10^{-7}$%) had no adverse effect [38]. Likewise, the indirect cytotoxicity is observed when dentin slabs are conditioned with 2% CHX [38]. Moreover, several *in vitro* studies indicated that exposure of human dentin to irrigation protocols containing 2% CHX could negatively impact the survival and attachment of DPSCs and SCAPs [24, 28]. However, when compared to EDTA and NaOCl, CHX have the lowest cytotoxic effect [32].

## Citric Acid

Citric acid is a chelating agent, with a potent anti-bacterial effect, and used as conditioning agent for dental root canal [53, 54]. Citric acid is as effective as EDTA in removing the inorganic component of smear layer and decalcification of dentin [53].

In regenerative endodontics, Hristov et al. suggested that 10% citric acid can be used in combination with 1.5% NaOCl, since there is no statistically significant difference between the effect of 10% citric acid and 17% EDTA on the vitality of SCAPs [35]. Moreover, Chae et al. found that 10% citric acid is efficient for releasing TGF-β1 *in vitro* with more biocompatibility than EDTA [36]. Recent studies showed that 10% citric acid conditioning is evidently more potent than 17% EDTA in releasing TGF-β1 [55, 56]. Consequently, citric acid have a significant high effect on stem cells migration, attachment, and survival [56]. These results contradicts with other studies showing that TGF-β1 release is significantly lower after treatment of dentin with citric acid compared to EDTA [49, 57].

## Combination of Irrigants

The use of EDTA as single irrigant is not sufficient to disinfect the root canal, therefore, it is recommended to use a sequence

**Table 1** The effect of the most used irrigants in regenerative endodontics on dental stem cells

| Researcher | Stem cells | Irrigant | Result of the study |
|---|---|---|---|
| Ring et al. 2008 [24] | DPSCs | − 6% NaOCl.<br>− 2% CHX.<br>- MCJ.<br>- EDTA. | - The root canals irrigated with 6% NaOCl and 2% CHX showed the lowest average numbers of attached DPSCs.<br>- MCJ/EDTA irrigation help maintain the survival and attachment of DPSCs. |
| Galler et al. 2011 [27] | DPSCs | − 5.25% NaOCl.<br>− 17% EDTA. | - NaOCl treatment followed by EDTA enhanced DPSCs attachment to dentin surface and differentiation into odontoblasts-like cells. |
| Trevino et al. 2011 [28] | SCAPs | − 17% EDTA.<br>− 6% NaOCl/17% EDTA/6% NaOCl.<br>− 17% EDTA/2% CHX.<br>− 6% NaOCl/17% EDTA/6% NaOCl/isopropyl alcohol/2% CHX. | - The irrigation with 17% EDTA is best supported SCAPs viability.<br>- Irrigants that included 2% CHX lacked any viable SCAPs.<br>− 17% EDTA promoted SCAPs survival and attachment to the root canal dentinal walls. |
| Martin et al. 2014 [18] | SCAPs | − 0.5%, 1.5%, 3%, or 6% NaOCl.<br>− 17% EDTA. | - High concentrations of NaOCl (3% and 6%) has a negative effect on the survival and differentiation of SCAPs.<br>- The negative effect is prevented with the use of lower concentration of NaOCl (0.5% or 1.5%) followed by 17% EDTA. |
| Pang et al. 2014 [29] | DPSCs | - EDTA | - EDTA induced DPSCs attachment and odontoblastic/osteoblastic differentiation. |
| Park et al. 2015 [30] | DPSCs | − 5.25% NaOCl.<br>− 1 mg/mL $Ca(OH)_2$.<br>− 17% EDTA. | - DPSCs did not attach to the dentin treated only by NaOCl.<br>- NaOCl treatment followed by Ca(OH)2 and EDTA promoted DPSCs attachment and differentiation. |
| Galler et al. 2016 [31] | DPSCs | − 5.25% NaOCl<br>− 10% EDTA | - NaOCl prohibited DPSCs attachment.<br>- EDTA conditioning induced the adhesion, migration and differentiation of DPSCs towards or onto dentin. |
| Farhad Mollashahi et al. 2016 [32] | SCAPs | − 5.25% NaOCl.<br>− 17% EDTA.<br>− 2% CHX. | - CHX have the lowest cytotoxicity compared to EDTA and NaOCl. |
| Zeng et al. 2016 [33] | DPSCs | − 1.5% NaOCl + 17% EDTA.<br>− 2.5% NaOCl + 17% EDTA.<br>− 17% EDTA. | − 17% EDTA group have significantly higher release of TGF-β1 than others groups (1.5% NaOCl + 17% EDTA and 2.5% NaOCl + 17% EDTA).<br>- The growth factors released into root canal space induced DPSCs migration. |
| Liu et al. 2018 [34] | DPSCs PDLSCs GMSCs | - NaOCl. | - The viability, proliferation and differentiation of dental stem cells are NaOCl dose-dependent. |
| Hristov et al. 2018 [35] | SCAPs | − 1.5% NaOCl / 17% EDTA.<br>− 1.5% NaOCl / 10% citric acid. | - The effect of 10% citric acid and 17% EDTA on the vitality of SCAPs is comparable. |
| Chae et al. 2018 [36] | SCAPs | − 1.5% NaOCl.<br>− 17% EDTA.<br>− 10% citric acid. | − 10% citric acid released the greatest amount of TGF-β1 compared to 17% EDTA, with significant biocompatibility regarding SCAPs viability. |
| M. El Shafei et al. 2018 [37] | DPSCs | − 5.25% NaOCl<br>− 5.25% NaOCl/17%EDTA<br>- MCJ<br>- MCJ/17% EDTA | - MCJ/EDTA combination promoted DPSC attachment to root canal dentin. |
| Widbiller et al. 2019 [38] | SCAPs | - CHX.<br>- EDTA. | - High concentration of CHX induced a significant decrease in SCAPs viability.<br>- EDTA did not decrease the CHX toxicity. |

of irrigants that are able to remove the smear layer, penetrate into infected dentinal tubules, and eradicate the bacteria [58].

Martin et al. suggested that 1.5% of NaOCl followed by 17% of EDTA promoted maximal survival and differentiation of SCAPs [18]. Moreover, a high concentration of NaOCl reaching 6%, followed by 17% EDTA, showed an acceptable surviving rate of SCAPs (74%) [28]. Recently, Zeng et al. indicated that 1.5% and 2.5% of NaOCl + 17% EDTA, released significantly higher amount of TGF-β1 compared 17% EDTA when used alone [33]. The logical reason for these results is that NaOCl is able to remove the organic components of smear layer, which leads to opening of dentinal tubules [27, 59].

Otherwise, the high cytotoxicity of CHX prevents dentists from using it as the final irrigant in REPs [42]. In 2015, Galler et al. noticed that irrigation with 0.12% CHX, for 5 min before EDTA conditioning, increased TGF-β1 release [49]. This is due to the fact that CHX acidity improved the dentin

demineralization [60]. On the other hand, the addition of 2% CHX after NaOCl + EDTA or EDTA alone, leads to loss of SCAPs viability [28].

### Morinda Citrifolia Juice (MCJ)

MCJ is a fruit-bearing tree belonging to the coffee family, which is broadly applied in pharmacology and medicine, since it has a wide range of therapeutic effects, including antiviral, antibacterial, antifungal, antitumor, anti-inflammatory, analgesic, hypotensive, immune-enhancing effects, and periodontal tissue regeneration activities [61]. In endodontics treatment, MCJ efficacy, as a natural intracanal irrigating solution, is similar to NaOCl, and superior to CHX, in removing up to 80% of the smear layer [62]. In REPs, many studies reported that MCJ/EDTA combination is the most optimal irrigant solution to help promoting the survival and attachment of DPSCs to the root canal walls [24, 37].

## Intracanal Medication

The inflamed immature permanent teeth are exposed to various bacterial colonies, including aerobic and anaerobic bacteria, where the use of intracanal medicaments is more frequent to disinfect the canal and avoid the development of antibiotic resistance [6]. Since the presence of periapical inflammation and intracanal bacteria can alter the osteogenic differentiation potential of stem cells [63], and can substantially impact the success of REPs [64], the intracanal medicaments are widely used in REPs due to their excellent disinfection effect [42]. Different studies recommended that the concentrations of intracanal medicaments used in REPs should be bactericidal but harmless to the host's cells, while having minimum effects on stem cells survival, proliferation, and attachment to canal walls (Table 2) [65]. A wide array of medicaments were assessed, where, the most commonly used medications are antibiotic pastes, such as triple antibiotic paste (TAP) (ciprofloxacin/metronidazole/minocycline), double antibiotic paste (DAP) (ciprofloxacin/metronidazole), and calcium hydroxide ($Ca(OH)_2$) [66]. 80% of the clinical articles used a combination of antibiotics as an intracanal medicament [26]. The most widely used combination is known as triple antibiotic paste (TAP), formulated of ciprofloxacin, metronidazole, and minocycline [5]. Various studies recommend the substitution of TAP by double antibiotic paste (DAP) to prevent the dentin discoloration effect of minocycline [67]. Moreover, $Ca(OH)_2$ is a strong base (pH 12.5–12.8), which eliminates the bacteria from the infected root canal when in direct contact with it [68]. It was demonstrated that TAP, DAP, and $Ca(OH)_2$ have the same effect on intratubular decontamination against *Enterococcus faecalis* biofilm [69, 70].

### Triple Antibiotic Paste (TAP)

Hoshino et al. was the first to develop TAP using a mixture of ciprofloxacin, metronidazole and minocycline (100 μg/ml each) [77]. TAP can be efficiently used to eliminate bacteria from the root canal system and plays a critical role in success of REPs [78]. TAP is used in 86% of REPs case reports [79]. In 2011, Lovelace et al. demonstrated that after disinfection of the canal space with TAP, the accumulation of undifferentiated stem cells from periapical region, contribute to the regeneration of pulpal tissues [11]. TAP was shown to provide an optimal antimicrobial effect, and create an appropriate environment for the stem cells attachment [80]. Moreover, many studies found that the use of low TAP concentrations (0.1 mg/mL to 1 mg/mL) exert the maximal effectiveness against *Enterococcus faecalis* biofilm, and the minimal side effects on the viability, attachment and proliferation abilities of SCAPs and DPSCs [71, 72, 81, 82]. Furthermore, Ruparel et al. indicated that concentrations greater than 0.1 g/mL of TAP are directly toxic to stem cells [65].

### Double Antibiotic Paste (DAP)

Given that the main treatment complication, reported by 40% of REPs studies, following the use of TAP was crown discoloration [83], various studies recommended the substitution of TAP by DAP (metronidazole and ciprofloxacin) [67]. Iwaya et al. was the first to reported the use of DAP as an intracanal medicament in a clinical case of REPs [84]. Within an infected root canal of an immature tooth, DAP (5 mg/mL) displayed significant direct antibacterial effects regardless of the bacterial biofilms [85]. Furthermore, 1 mg/mL of DAP showed a significant antibacterial effect against *Enterococcus faecalis* and *Prevotella intermedia* biofilms, without provoking a significant negative effect on viability, proliferation and mineralization of DPSCs [69]. On the other hand, Kim et al. found that a 1 mg/mL concentration of DAP, followed by EDTA irrigation for 10 min, caused significant increase in the DPSCs attachment and proliferation ability [74].

### Calcium Hydroxide ($Ca(OH)_2$)

$Ca(OH)_2$ is an antimicrobial agent which can stimulate the hard tissue repair in endodontics treatment [86]. The alkaline PH of $Ca(OH)_2$ reduced osteoclasts activity, induced a bactericidal effect, and can activate alkaline phosphatases reaction which play a critical role in hard tissue formation [87]. Furthermore, $Ca(OH)_2$ is tolerated by bone and dental pulp tissues [86]. $Ca(OH)_2$ is used in 13% of REPs case reports [79]. Several studies showed that dentin conditioning with $Ca(OH)_2$ is not toxic at all tested concentrations, and it can stimulate SCAPs survival and proliferation [72, 88]. Moreover, recent studies showed that $Ca(OH)_2$ is able to significantly improve the mineralization capacity and the osteogenic differentiation of DPSCs [69, 75]. Ruparel et al. showed

**Table 2** The effect of the most used medicaments in regenerative endodontics on dental stem cells

| Researcher | Stem cells | Medicaments | Result of the study |
|---|---|---|---|
| Ruparel et al. 2012 [65] | SCAPs | - TAP.<br>- DAP.<br>- $Ca(OH)_2$. | - High concentrations of TAP and DAP have a negative effect on SCAPs survival, whereas lower concentrations are conducive with SCAPs viability and proliferation.<br>- $Ca(OH)_2$ is conducive with SCAP survival at all concentrations. |
| Chuensombat et al. 2013 [71] | DPSCs<br>SCAPs | - TAP. | - The cytotoxicity of TAP is concentration- and time-dependent.<br>– 0.39 μg/mL of TAP have less cytotoxicity on stem cells and is able to significantly reduce bacteria. |
| Althumairy et al. 2014 [72] | SCAPs | – 1 mg/mL or 1000 mg/mL TAP.<br>– 1 mg/mL or 1000 mg/mL DAP.<br>- $Ca(OH)_2$. | - High concentrations of TAP or DAP (1000 mg/mL) resulted in no viable SCAPs, whereas the use of these antibiotics at low concentration (1 mg/mL) exerted no negative effect on SCAPs viability.<br>- $Ca(OH)_2$ treatment significantly increased SCAPs survival and proliferation. |
| Sabrah et al. 2015 [73] | DPSCs | - TAP.<br>- DAP. | - All antibiotics concentrations have an antibacterial effect against Enterococcus faecalis biofilm.<br>- Low concentrations of DAP and TAP showed a significant antibacterial effect with no cytotoxic effects on the viability of DPSCs. |
| Kim et al. 2015 [74] | DPSCs | - DAP<br>- EDTA | - High concentrations of DAP decreased the DPSCs proliferation, whereas low concentrations did not show a negative effect on DPSCs proliferation.<br>- DAP treatment followed by EDTA irrigation caused significant increases in DPSCs attachment compared to treatment with the DAP alone. |
| Chen et al. 2016 [75] | DPSCs | - $Ca(OH)_2$. | - $Ca(OH)_2$ induces proliferation, migration, mineralization, and osteogenic differentiation of the DPSCs. |
| Alghilan et al. 2017 [76] | DPSCs | - TAP.<br>- DAP.<br>- $Ca(OH)_2$. | - TAP, DAP and $Ca(OH)_2$ caused significant reduction in DPSC proliferation.<br>- TAP caused significant increases in DPSC attachment to dentin. |
| McIntyre et al. 2019 [69] | DPSCs | - DAP.<br>- $Ca(OH)_2$. | - $Ca(OH)_2$ and 1 mg/mL of DAP showed a significant direct antibacterial effect without causing significant negative effects on the proliferation of DPSCs.<br>- $Ca(OH)_2$ significantly induced the mineralization capacity of DPSCs. |

that high concentrations of intracanal antibiotics have a negative effect on SCAP survival; whereas, all concentrations of $Ca(OH)_2$ are conducive with SCAPs survival and proliferation [65].

### Comparison Among Medicaments

Many studies compared the stem cells fate among multiple medicaments in REPs. Sabrah et al. reported that 0.125 mg/ml of TAP and DAP significantly reduced the *Enterococcus faecalis* biofilm with no cytotoxic effects on the viability of DPSCs [73]. Vice versa, high concentrations of TAP or DAP (1000 mg/mL) altered SCAPs viability [72]. According to a recent study, TAP induced less cytotoxic effect against SCAPs and it is the safest antibiotic, compared to DAP and $Ca(OH)_2$ [89]. Alghilan et al. indicated that TAP enhanced the attachment of DPSCs to the dentin, while, TAP, DAP, and $Ca(OH)_2$ reduced DPSCs proliferation [76].

## Molecular Mechanisms of Regenerative Endodontics Procedures

REPs bioengineering therapies used regenerative features of stem cells to heal periapical lesions and recuperate pulp-dentin complex in immature permanent teeth [90]. These procedures involve a triad of conditions: disinfection, accumulation of stem cells into scaffolds containing growth factors, and coronal restoration to prevent future infection (Fig. 2) [91]. Moreover, the three main components needed in REPs are stem cells, growth factors and scaffolds, that can initiate and promote the differentiation of cells into pulp-dentin complex [27]. The generation of distinct pulp-dentin complex in REPs may have been stimulated by specific exogenous or endogenous stem cells and growth factors [92]. The identification of released growth factors and other bioactive molecules, stimulated by the various canal irrigants and medicaments used in endodontics, provides the opportunity to identify the key signaling molecules promoting improvement of REPs [93]. Dentin contains a considerable number of signaling molecules reserved in its mineralized matrix [94]. However, the dentin disinfection agents and medicaments used during REPs should allow the release of these signaling molecules from dentin [48], and provide a convenable microenvironment to stem cells survival, attachment, migration, and differentiation [24, 65]. In addition, an adequate scaffold should be provided to let released signaling molecules be accessible to dental stem cells [95]. Various studies explored the signaling

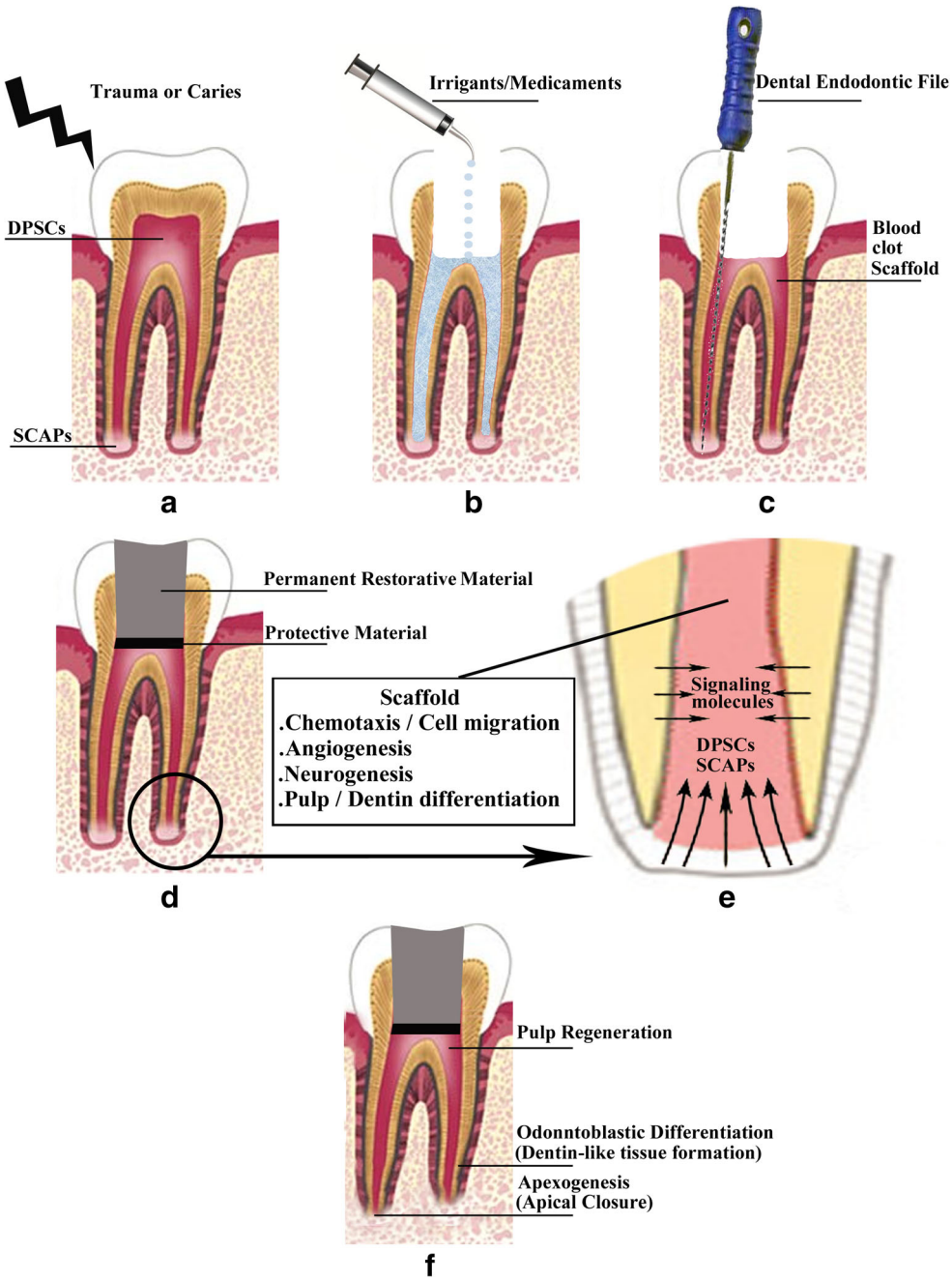


**Fig. 2** Schema illustrating the potential target of irrigants and medicaments in REPs. **A**. Immature permanent teeth exposed to trauma or caries. **B**. Access cavity preparation and chemical debridement by using of irrigants and/or medicaments. **C**. Bleeding induced by over-instrumentation to create a three-dimensional scaffold. **D**. Restoration of the access cavity with a protective material covering the scaffold, followed by coronal-tight permanent restoration to prevent future reinfection. **E**. Release of signaling molecules (growth factors) sequestered in dentin, and their influences on SCAPs (from apical papilla) and remaining DPSCs, including chemotaxis/cell migration, angiogenesis, neurogenesis, and differentiation into pulp/dentin complex. **F**. Achievement of tissue regeneration (apexogenesis/maturogenesis) determined by continued root development, increased dentinal wall thickness by dentin-like deposition, and apical closure

molecules released from dentin and showed that they have diverse effects [93, 94, 96], such as:

- Chemotaxis/cell migration: interleukin-8 (IL-8), and transforming growth factor β-1 (TGFβ-1).
- Angiogenesis: vascular endothelial growth factor (VEGF).
- Neural growth: glial cell line-derived neurotrophic factor, and brain-derived neurotrophic factor (BDNF).
- Proliferation: fibroblast growth factor-2 (FGF-2), and Insulin growth factor-1 (IGF-1).
- Differentiation: TGF-β1, IGF-1, and Bone morphogenetic protein 2 and 4 (BMP-2 and BMP-4).

In 1992, Begue-Kirn et al. reported that bone morphogenetic protein 2 (BMP-2), which is a growth factor present in dentin, stimulate the differentiation of dental derived stem cells into odontoblasts [97]. Casagrande et al. demonstrated that dentin-derived BMP-2 induces SHEDs differentiation into odontoblasts [98]. Controversially, dentin formation is markedly reduced without BMP-2 and BMP-4 [99]. An *in vivo* study announced that FGF led to recellularization

and re-vascularization of the root canals [100]. On the other hand, an *in vitro* study reported that FGF-2 could induce DPSCs proliferation, while TGFβ-1 or (FGF2 + TGFβ-1) stimulated the odontoblastic differentiation of DPSCs [101]. Moreover, IGF-1 can promote the proliferation and differentiation of DPSCs into a mineralized tissue [102]. Besides, Iohara et al. found that DPSCs and granulocyte-colony stimulating factor (G-CSF) induced the regeneration of pulp and new dentin [103, 104]. Furthermore, (FGF and/or VEGF) combined with nerve growth factor (NGF) and BMP-7, produced recellularized and revascularized connective tissue and new dentin over the surface of native dentinal wall in endodontically treated root canals [105].

Since the use of a single disinfectant molecule is not sufficient, the REPs suggest the use of a combination or sequence of irrigants and/or medicaments, that are able to disinfect the canal environment, remove the smear layer, and enhance the release of growth factors [106]. The last disinfectant which conditioned the dentin should expose the growth factors from dentinal tubules, and stimulate the stem cells differentiation when they are in contact with this dentin [48]. It was shown that EDTA treatment exposed TGF-β1 on the dentin surface, while citric acid and NaOCl revealed lesser quantity [57]. Galler et al. suggested that EDTA-conditioned dentin induced the release of TGF-β1, FGF-2, and VEGF after irrigation with CHX followed by 10% EDTA; whereas NaOCl attenuate this effect [49]. These previous studies are in line with results reported by Galler et al. that dentin conditioning by EDTA as final irrigant in REPs enhanced the migration and attachment of DPSCs towards or onto dentin, and stimulated their differentiation into odontoblast-like cells [31]. Based on the previously mentioned studies, conditioning of the dentin with EDTA as final rinse is beneficial for achieving successful outcomes in REPs [29]. Furthermore, the intracanal medicaments interfere with the release of growth factors when they are retained in the root canal dentin surfaces [49]. $Ca(OH)_2$ irrigation followed by EDTA conditioning, were able to release a high amounts of TGF-β1, due to the fact that $Ca(OH)_2$ can be removed from dentin [49]; whereas TAP and CHX are retained in the root canal dentin [107].

Despite of all these observations, further studies are needed to elucidate the exact molecular mechanisms underlying these effects.

## Conclusions

While more *in vivo* studies and clinical research are needed, the regenerative endodontics have developed a new insight for treating infected immature permanent teeth, by allowing tissue regeneration and repair to achieve apexogenesis/maturogenesis. This enabled to avoid performing apexification procedures.

Overall, the comprehensive knowledge on the effect of irrigation on dental pulp regeneration, which results from the present literature review, deals with:

- The validity of some irrigants employed in the analyzed studies [24]. Additional improvements such as use of natural intracanal irrigating solution (e.g. Morinda Citrifolia Juice) are in progress, [62].
- The efficacy of irrigants in release of growth factors, especially when they are dentin-derived [25].
- The feasibility and safety of medicaments in mature teeth with inflamed pulp [67].

The *in vivo* and *in vitro* experiments cited in this review showed that multiple irrigants and medicaments, alone or in combination, have the potential to induce the release of many growth factors from the dentin; and they have biological effects on human DPSCs, SCAPs, and other stem cells, concerning their migration, proliferation and differentiation (Fig. 3). This is essential to achieve dental pulp regeneration.

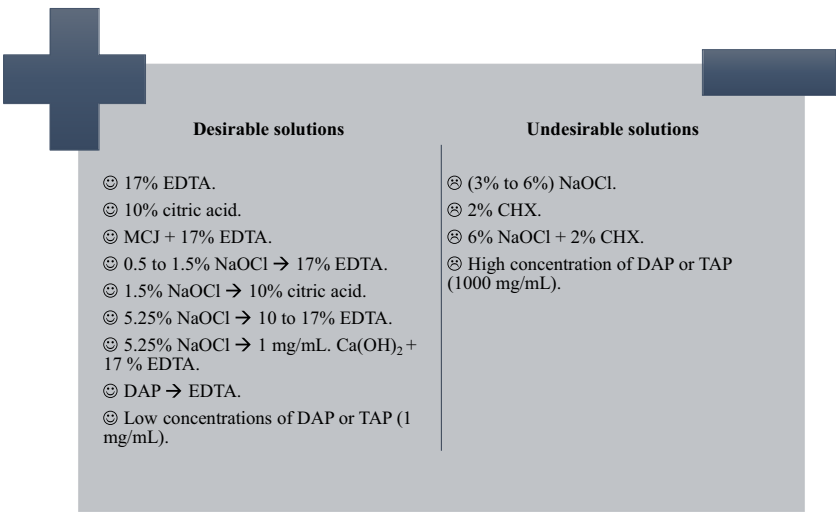


**Fig. 3** The irrigants and medicaments used in REPs, divided according to their influence on stem cells. (+ combined; ◊ sequential)

This present review did not estimate all the possible combinations of irrigants and medicaments and their effects on stem cells. Therefore, further researches are required to investigate other irrigants and medicaments and should focus on the estimation of their concentrations and time of use.

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## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

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