# Translational Science in Disinfection for Regenerative Endodontics

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## Abstract

The endodontic management of permanent immature teeth is fraught with challenges. Although treatment modalities for vital pulp therapy in these teeth provide long-term favorable outcome, the outcomes from the treatment of pulp necrosis and apical periodontitis are significantly less predictable. Immature teeth diagnosed with pulp necrosis have been traditionally treated with apexification or apexogenesis approaches. Unfortunately, these treatments provide little to no benefit in promoting continued root development. Regenerative endodontic procedures have emerged as an important alternative in treating teeth with otherwise questionable long-term prognosis because of thin, fragile dentinal walls and a lack of immunocompetency. These procedures rely heavily on root canal chemical disinfection of the root canal system. Traditionally, irrigants and medicaments have been chosen for their maximum antimicrobial effect without consideration for their effects on stem cells and the dentinal microenvironment. Translational research has been crucial to provide evidence for treatment modifications that aim to increase favorable outcome while steering away from common pitfalls in the currently used protocols. In this review, recent advances learned from translational research related to disinfection in regenerative endodontics are presented and discussed. *(J Endod 2014;40:S52–S57)*

## Key Words

Disinfection, immature teeth, mesenchymal adult stem cells, pulp biology, regenerative endodontics, revascularization, root canal disinfection

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The developing dentition is at risk for pulpal necrosis because of trauma and developmental dental anomalies such as dens evaginatus (1–7). The loss of an immature permanent tooth in young patients with mixed dentition can be devastating, leading to loss of function, malocclusion, and inadequate maxillofacial development. These teeth traditionally have been treated with apexification procedures using either long-term calcium hydroxide treatment (8, 9) or immediate placement of a mineral trioxide aggregate (MTA) apical plug (10). Although these treatments often result in the resolution of signs and symptoms of pathosis, they provide little to no benefit for continued root development (11). Thus, immature teeth treated with these procedures are considered to be in a state of "arrested development," and no further root growth, normal pulpal nociception, and immune defense should be expected.

Regenerative endodontic procedures (REPs) have emerged as an alternative treatment alternative for these teeth that, in addition to healing of apical periodontitis, aims to promote normal pulpal physiologic functions. These include continued root development, immunocompetency, and normal nociception, as seen in some published cases (12). Thus, the ultimate goal of these procedures is to regenerate the components of the pulp-dentin complex. A significant number of case reports and case series have been published since the first reported case in 2001 (12). These published cases document the following (12):

1. Commonly observed clinical outcomes such as continued root development and sometimes normal nociceptive responses to vitality testing
2. Commonly found challenges such as technical pitfalls and unwanted adverse reactions such as coronal staining
3. Great variability in treatment protocols

Despite the lack of randomized clinical trials, these published clinical observations support the hypothesis that patients with otherwise limited treatment options could benefit from these procedures.

In 2011, a study showed that a substantial number of undifferentiated mesenchymal stem cells are delivered into root canal systems after REPs (13). This finding represented a turning point because treatment protocols previously used in REPs aimed to provide maximum disinfection without consideration for their impact on stem cells. Contemporary regenerative endodontics acknowledges and follows principles of bioengineering regarding the interplay between stem cells, scaffolds, and growth factors (14). Because stem cells represent 1 of the pillars of REPs, a series of translational studies evaluating the effect of disinfection on stem cell fate have been conducted. These studies have contributed to the foundational framework for the current American Association of Endodontists–recommended regenerative endodontic treatment protocol (15).

## Translational Studies on Disinfection

### Irrigants

Clinicians often face the challenge of adequately debriding large infected root canals in REPs. In these procedures, similar to conventional endodontic therapy, microbial control is crucial. These canals with compromised fragile underdeveloped dentinal walls represent a contraindication for mechanical instrumentation; thus, chemical debridement remains the main form of disinfection in REPs. Sodium hypochlorite (NaOCl) is the most widely used agent for chemical debridement in endodontic procedures, including REPs (12). It has several desirable characteristics including excellent bactericidal efficacy (16–18), tissue dissolution capacity (19–21), and effective

lubrication for endodontic instruments. The first 2 beneficial properties are crucial for the disinfection of immature teeth in REPs, which typically involve minimal to no mechanical preparation.

A study evaluated the survival of stem cells of apical papilla (SCAPs) cultured in an organotype root canal model previously irrigated with various combinations of commonly used chemical agents (22). It was found that dentin conditioning with 17% EDTA promoted greater survival of SCAP, whereas the use of 6% NaOCl had a profound detrimental effect on SCAP survival. Importantly, the use of EDTA after 6% NaOCl attenuates its undesirable effects (22). Independent studies have shown that dentin conditioning with 5%–6% NaOCl prevented differentiation of SHED and dental pulp stem cells (DPSCs) into an odontoblastlike phenotype in both *in vitro* (23) and *in vivo* models (23, 24). Moreover, this prolonged effect of dentin conditioning with NaOCl persisted long after the irrigant had been removed, suggesting that NaOCl has both direct and indirect effects on stem cell toxicity (25). Thus, dentin conditioning with NaOCl at its maximum clinically used concentration leads to greatly diminished stem cell survival and loss of odontoblastlike cell differentiation.

A recent study was conducted to evaluate whether other clinically used concentrations of NaOCl were conducive for stem cell (SCAP) survival and differentiation (26). Standardized root canals were prepared in extracted human teeth. The prepared teeth were then irrigated with NaOCl at concentrations of 6%, 3%, and 1.5%. Approximately half of the samples received a second irrigation with 17% EDTA, whereas all samples received a copious final flush with saline to remove any residual chemical from the canal space. SCAPs in a hyaluronic acid hydrogel were seeded in all canals and cultured for 7 days. The number of viable cells was assessed using a luminescent assay, whereas the level of dentin sialophosphoprotein was assessed by quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR). It was found that dentin conditioning with NaOCl decreases both SCAP survival and differentiation in a concentration-dependent manner. However, the concentration of 1.5% of NaOCl was found to have minimal effects on survival and differentiation (Fig. 1*A* and *B*). This study agrees with other studies that dentin conditioning with 6% NaOCl has a negative effect, whereas 17% EDTA has a positive effect on the survival and differentiation of stem cells subsequently cultured in contact with the conditioned dentin (22, 26, 27). Furthermore, the use of EDTA as a last step in the irrigation protocol partially reversed the detrimental effects of NaOCl. The negative effects of NaOCl do not appear to be directly related to residual NaOCl in the dentinal tubules, resulting in direct toxicity because neutralization with sodium thiosulfate (5%) did not reverse this effect (26). Thus, NaOCl has a profound effect on dentin, resulting in diminished stem cell survival and differentiation. These effects can be minimized by using 1.5% NaOCl followed by 17% EDTA (19).

Important biologically active growth factors are trapped in the dentin matrix during dentinogenesis. Some of these growth factors such as vascular endothelial growth factor (28) and transforming growth factor beta 1 (29) are known to have a robust effect on the differentiation and/or proliferation of mesenchymal stem cells. These growth factors appear particularly efficacious in promoting the proliferation of mesenchymal stem cells and directing them toward an odontoblastlike phenotype (30, 31). Irrigants, especially NaOCl in high concentrations, are known to denature these dentin-derived growth factors (32). In an *in vivo* study, DPSCs proliferated at higher rates and expressed higher levels of odontoblastic markers in a tooth slice model compared with DPSCs placed in a scaffold only (27). These findings suggest that morphogens, such as the many growth factors known to be present in dentin, are sufficient to promote survival, proliferation, and, importantly, differentiation of dental stem cells. EDTA is known to solubilize these growth factors from dentin, thereby increasing their bioavailability (33, 34). Thus, its use may allow clinicians to harness the inductive properties of dentin-derived morphogens and growth factors normally present in dentin (35).

Stem cell proliferation and differentiation are also known to be dictated by the surface on which the cells grow (36–38). Stem cells attach to a specific surface such as a target organ during organogenesis, or repair, via the interaction of specific cell-adhesion molecules such as integrins expressed on the plasma membrane of these cells. The effect of the substrate on stem cell behavior is best shown by the effect of the stem cell niche that, in addition to growth factors (discussed previously), provides an attachment signal, resulting in cell arrestment on a quiescent state (39, 40). Cells released from their niche become "activated" and start proliferating and undergoing differentiation. The process of culturing tooth-derived stem cells such as DPSCs or SCAPs is a good example of cells leaving their inhibited state in the niche (dental pulp or apical papilla, respectively) and displaying remarkable proliferative and differentiation potentials. This information has strong clinical implications because the dentin matrix composition (stem cell substrate) is altered by chemical treatment during the

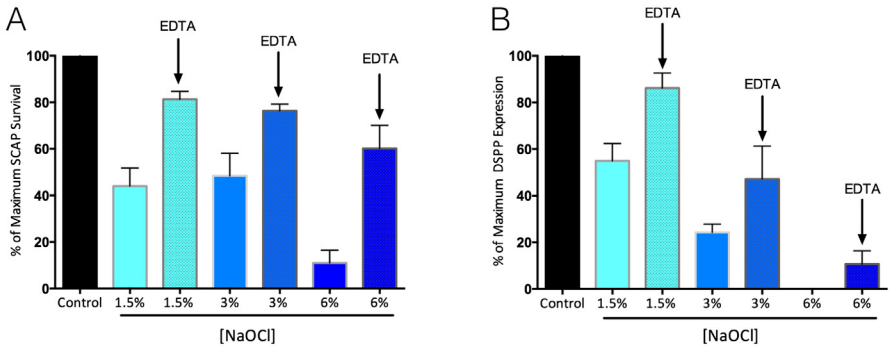


**Figure 1.** NaOCl decreases SCAP survival and differentiation in a concentration-dependent manner. Organotype immature teeth root canal models were irrigated with different concentrations of NaOCl following a standardized protocol that included a final wash of saline or EDTA. SCAPs were seeded into the root segments and cultured *in vitro* for 7 days. The percentage of viable cells was determined by a luminescence assay (CellTiter-Glo; Promega, Madison, WI). (*A*) The NaOCl concentration-dependent decrease in SCAP survival is partially reversed by a final irrigation with 17% EDTA. In addition, real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to determine the expression of the odontoblastlike cell marker dentin sialophosphoprotein (DSPP) messenger RNA. NaOCl decreases DSPP expression in a concentration-dependent manner with no expression observed in the group treated with 6%. (*B*) In addition, EDTA partially reversed the negative effect of NaOCl on DSPP expression. Data are presented by the percentage of the maximum observed effect on the EDTA-only treatment group (control). Data are presented as mean ± standard error of the mean. (Modified version of the published data from Martin DE, De Almeida JF, Henry MA, et al. Concentration-dependent effect of sodium hypochlorite on stem cells of apical papilla survival and differentiation. J Endod 2014;40:51–5.)

process of chemical debridement. NaOCl is known to cause changes in dentin matrix composition with a decrease in carbon and nitrogen content and demineralization when used at high concentrations (41). In contrast, the concentration of 1% NaOCl did not cause any significant changes in dentin composition or mechanical properties. Thus, changes in dentin chemical composition could interfere with the ability of stem cells to attach, proliferate, and differentiate on the dentin surface. Indeed, a previous study found that NaOCl greatly impacted DPSC attachment on dentin, whereas EDTA promoted attachment (42) and stem cell differentiation (23, 24). Thus, hypochlorite is likely to affect the fate of stem cells by altering the dentin chemical composition, including the denaturation of growth factors and attachment molecules.

## Intracanal Medicaments

Intracanal medicaments have been used in all published REPs with the exception of 1 case report (12, 43). Approximately half of the published regenerative endodontic cases were treated with an antibiotic paste as an intracanal medicament. The use of antibiotic formulations as endodontic medicaments has been documented many decades before the first revascularization report (44, 45). An antibiotic mixture composed of ciprofloxacin, metronidazole, and minocycline, known as triple antibiotic paste (TAP) or "3mix," has been the most widely used medicament. This antibiotic formulation was first tested *in vitro* against bacteria isolated from carious lesions and endodontic infections in deciduous teeth (46). It was found that no bacteria could be recovered after treatment with 100 $\mu$g/mL of each antibiotic (300 $\mu$g/mL of mixture) (46). Next, studies were performed to test the efficacy of TAP in eradicating bacteria from infected dentin. There were no recovered bacteria by 48 hours, and evidence was found of significant drug dentin penetration (46). Therefore, Hoshino et al used this combination of ciprofloxacin, metronidazole, and minocycline dissolved in a macrogol/propylene glycol vehicle to disinfect infected deciduous teeth by placing it at the orifice followed by diffusion into the canal space and associated tissues, a procedure they called "lesion sterilization and tissue repair therapy" (46, 47). A total of 87 infected deciduous teeth were treated with this protocol, resulting in the resolution of symptoms within days, with teeth remaining asymptomatic until the exfoliation and eruption of successor permanent teeth (47). Interestingly, the concentration of 100 $\mu$g/mL of each drug was sufficient to completely eradicate cultivable bacteria from infected root canals *in vitro* and in clinical samples (46, 47). Furthermore, in an independent study, the application of TAP in immature dog teeth for 2 weeks resulted in 70% of all canals being free of cultivable microorganisms, and the remaining detectable bacteria were present in extremely small levels (48). Collectively, these data suggest that this antibiotic combination is highly effective against endodontic microorganisms.

Based on this prior research, TAP was used in a regenerative endodontic procedure for the first time in 2004 (49). It quickly became the most commonly used intracanal medicament (12). The drugs were mixed with water, saline, or propylene glycol until a thick creamy mixture was formed. It is important to highlight that in these case reports there was no attempt to deliberately deliver a specific concentration of the drugs. Instead, drugs were mixed until a certain physical consistency was achieved that was deemed suitable by clinicians. However, it is important to appreciate that any pharmacologic agent has a therapeutic window that varies from an ineffective minimal concentration that is devoid of therapeutic efficacy to excessive concentrations that lead to unwanted side effects and/or toxicities. Thus, an ideal concentration of the antibiotic mix would be maximally effective against bacteria while being harmless to the host's cells, including stem cells. This concept is potentially relevant to REPs because safe therapeutic circulating levels of these antibiotics in humans are at concentrations of about 0.001–0.01 mg/mL, whereas "pastelike" formulations are at levels of about 1000 mg/mL, a difference in concentration of up to a million-fold.

To address this issue, a recent study evaluated the concentration-dependent effect of various intracanal antibiotic combinations (including TAP) on the survival of SCAPs *in vitro* (50). At concentrations providing a pastelike consistency, there was similar toxicity for all other combinations of antibiotic tested such as double antibiotic paste (ciprofloxacin and metronidazole), amoxicillin with clavulanic acid, and modified TAP (minocycline is substituted by cefaclor). In contrast, a commercial preparation of calcium hydroxide had no detrimental effect on SCAP survival (Fig. 2*A* and *B*). Importantly, this study found that antibiotic paste toxicity is greatly diminished when these drugs are used at concentrations of 0.1 mg/mL or 0.01 mg/mL; both

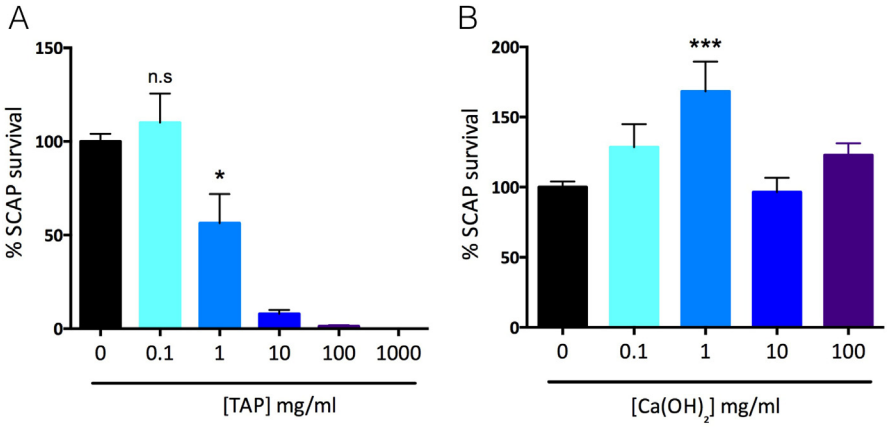


**Figure 2.** Intracanal medicaments commonly used in REPs have opposite effects on SCAP survival. Approximately 600,000 cells were plated in a 24-well plate and treated with media only or progressive 1:10 serial dilutions from the 1000-mg/mL stock of TAP or $Ca(OH)_2$ (Ultracal; South Jordan, UT). Cell counting was performed on day 3 or at 80% confluency using an automated method of detecting trypan blue dye ($n$ = 6–10, experiments were performed in triplicates). (*A*) TAP decreased SCAP viability in a concentration-dependent manner with $LC_{50}$ = 1 mg/mL and no SCAP viable at the concentration required for a pastelike consistency (1000 mg/mL). (*B*) In contrast, $Ca(OH)_2$ has no detrimental effect on SCAP survival with an increase in proliferation observed at the concentration of 1 mg/mL. Data are presented as mean ± standard error of the mean. *$P$ < .05. ***$P$ < .001. n.s., not statistically significant tested by 1-way analysis of variance. $LC_{50}$, concentration that elicits 50% mortality. (Modified from Ruparel NB, Teixeira FB, Ferraz CC, et al. Direct effect of intracanal medicaments on survival of stem cells of the apical papilla. J Endod 2012;38:1372–5.)

concentrations were previously found to be effective against endodontic bacteria (46, 47). These drugs are typically applied to the entire length of root canals for periods that vary from weeks (45, 49) to months (51). Therefore, they contact the residing apical papilla stem cells for a prolonged period, possibly leading to toxicity, and decrease the number of viable mesenchymal stem cells (MSCs). In addition, there is the potential for direct interaction of residual antibiotics remaining on dentin walls with stem cells delivered into the root canal system after the evoked bleeding step in REPs.

A follow-up study was conducted to evaluate whether TAP was efficiently removed from root canal systems using commonly used clinical irrigation techniques (52). A radiolabeling approach was used to tag TAP with the isotope $I^{135}$ and a $Ca(OH)_2$ paste with the isotope $C^{45}$. The labeled medicaments were placed into standardized root canals in extracted human teeth. After a period of 28 days, the canals were irrigated with either positive pressure, positive pressure aided ultrasonic activation (passive ultrasonic irrigation). Radioactivity was measured in the fluid collected after standardized irrigation protocols as well as residual radioactivity remaining in the tooth after completion of the irrigation protocol. In addition, the dentinal walls were sequentially instrumented followed by radioactivity measurements of the dentin shavings to determine the penetration of the labeled medicaments into dentin. Despite using different techniques with copious irrigation, <20% of TAP could be removed with the remaining 80% tightly bound to dentin (Fig. 3) to depths >350 $\mu$m (data not shown). Conversely, >85% of Ca(OH)2 could be removed with irrigation alone (Fig. 3), and the residual labeled Ca(OH)2 was found in dentinal depths <350 $\mu$m (data not shown). This high penetration and binding of TAP can be appreciated clinically as the substantial staining seen in dentin. Because bacteria are known to penetrate and colonize dentinal tubules at depths >350 $\mu$m (53), the high penetration of TAP may underlie its desirable antibacterial effects. Thus, differently than $Ca(OH)_2$, TAP has high penetration and binding into dentin. However, this property also increases the likelihood that MSCs delivered into the root canal system during REPs will have a direct contact with the residual medicament. Based on the studies discussed in this review, calcium hydroxide could be considered the first line of intracanal medication in REPs. Alternatively, antibiotic pastes such as TAP can be used as primary medicaments or after calcium hydroxide if the treatment dictates more aggressive disinfection as long as the concentration of the drug respects their therapeutic range (0.01–0.1 mg/mL) (46, 50).

In addition to the direct effect of these medicaments, they may have an indirect effect by altering dentin properties and thus altering stem cell fate. Indeed, some of these medications have been found to result in changes in the dentin composition sufficient to lead to changes in dentinal strength and resistance to fracture (54). Some of the changes observed are likely to result from the low pH of these drug combinations when suspended in water or saline, which approximates a pH of 3, resulting in dentin demineralization (55). It is important to highlight that prolonged treatment with $Ca(OH)_2$ formulations, with high pH levels (~12), also alters dentinal composition, resulting in increased susceptibility to fractures (54, 56). Therefore, besides changes in the dentin mechanical properties, alterations on the dentin matrix composition by intracanal medicaments could alter the fate of stem cells when contacting the modified substrate.

The effect of dentin treatment with TAP or $Ca(OH)_2$ was recently evaluated (57). SCAP survival and proliferation cultured on dentin previously treated with different concentrations of TAP or calcium hydroxide were measured. There were no viable SCAPs when cultured in contact with dentin conditioned with the highest concentration of TAP tested of 1000 mg/mL, which is equivalent to a pastelike consistency currently used in REPs. However, SCAPs cultured on dentin conditioned with 1 mg/mL TAP had survival equivalent to SCAPs cultured on dentin exposed to saline (control). Interestingly, SCAPs had the highest survival when cultured on dentin previously exposed to $Ca(OH)_2$ (57) (Fig. 4). These findings suggest that similar to irrigants, medicaments have the potential to alter the substrate (dentin) where ideally stem cells should proliferate and differentiate. The delicate balance between disinfection and stem cell fate warrants further research.

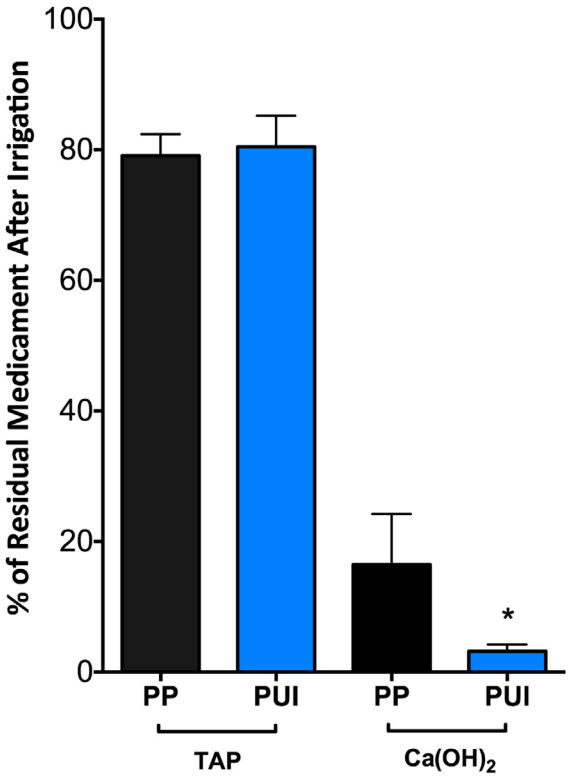


**Figure 3.** TAP remains in dentin, whereas most $Ca(OH)_2$ is eliminated after endodontic irrigation. Radiolabeled TAP or $Ca(OH)_2$ was placed within canals of standardized root segments and incubated for 28 days at 37°C. The canals were flushed with standardized volumes of EDTA and saline using either positive pressure with a side-vented needle (PP) or positive pressure with ultrasonic activation of irrigants (PUI). There was no difference in labeled TAP removal among groups with only approximately 20% of the medicament being removed by the irrigation protocols. In contrast, >80% of $Ca(OH)_2$ was removed, with more efficient removal observed in canals irrigated with PUI. Data are presented as the mean percentage of total radiolabeled medicament removal ± standard error of the mean. *$P$ < .05 tested by the Student $t$ test ($n$ = 12/group). (From Berkhoff J, Chen PB, Teixeira FB, et al. Effective removal of triantibiotic paste during regenerative endodontic treatment: an evaluation of different irrigation procedures. J Endod 2013;39:e15.)

## Overview of a Regenerative Endodontic Procedure

The following protocol reflects our current personal recommendations for regenerative procedures and is based on the best level of available evidence from clinical or preclinical translational studies. These recommendations are based in part on the dual requirement of selecting irrigants and medicaments at concentrations that are known to be effective against microorganisms while being least toxic to stem cells. It is important to recognize that these

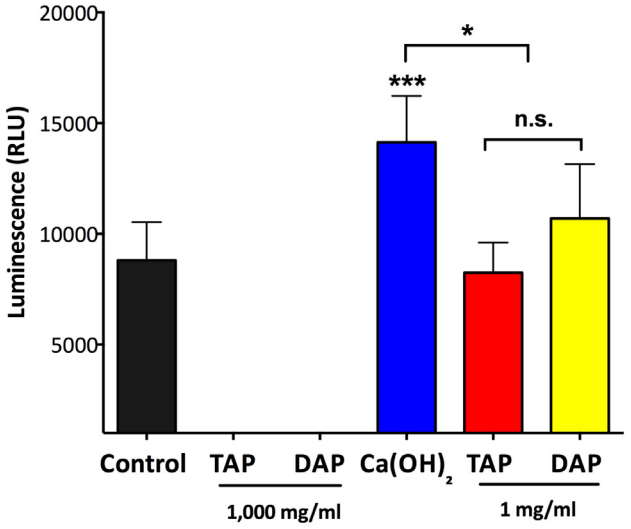


**Figure 4.** Dentin conditioning for 7 days with medicaments used in REPs has a profound effect on SCAP survival. Standardized dentin disks were treated for 7 days with TAP or double antibiotic paste (DAP) (concentrations of 1000 mg/mL or 1 mg/mL), $Ca(OH)_2$ (Ultracal), or sterile saline (control). SCAP in a Matrigel scaffold (BD Biosciences; Bedford, MA) was seeded into the lumen of the disks after the medicaments were removed and cultured for 7 days. Cell viability (survival) was determined using a luminescent assay. SCAP culture on dentin treated with TAP or DAP at the concentration of 1000 mg/mL resulted in no viable cells. Conversely, dentin conditioning with TAP or DAP at the concentration of 1 mg/mL supported cell viability with no difference from untreated dentin disks (control). Greater survival and proliferation were detected in the group treated with $Ca(OH)_2$. Data are presented as mean ± standard deviation of relative luminescence units ($n$ = 12/group). *$P$ < .05. ***$P$ < .001. n.s., no statistical difference as tested by 1-way analysis of variance.

recommendations are likely to change as the field of regenerative endodontics evolves.

## Treatment Procedures for Regenerative Endodontics

The following are performed during the first treatment visit:

1. Informed consent, including explanation of risks and alternative treatments or no treatment, is obtained.
2. After ascertaining adequate local anesthesia, rubber dam isolation is obtained.
3. The root canal systems are accessed, and the working length is determined (radiograph of a file loosely positioned at 1 mm from the root end).
4. The root canal systems are slowly irrigated first with 1.5% NaOCl (20 mL/canal for 5 minutes) and then irrigated with 17% EDTA (20 mL/canal for 5 minutes), with the irrigating needle positioned about 1 mm from the root end.
5. Canals are dried with paper points.
6. Calcium hydroxide or antibiotic paste at a concentration no greater than 1 mg/mL is delivered to the canal system.
7. Access is temporarily restored.

During the final (second) treatment visit (2–4 weeks after the first visit), the following are performed:

1. A clinical examination is first performed to ensure that that there is no moderate to severe sensitivity to palpation and percussion. If such sensitivity is observed or a sinus tract or swelling is noted, then the treatment provided at the first visit is repeated. At this point, the clinician may elect to use TAP (at no more than 100 $\mu$g of each drug/mL).
2. After ascertaining adequate local anesthesia with 3% mepivacaine (no epinephrine), rubber dam isolation is obtained.
3. The root canal systems are accessed; the intracanal medicament is removed by irrigating with 17% EDTA (30 mL/canal for 10 minutes).
4. The canals are dried with paper points.
5. Bleeding is induced by rotating a precurved K-file size #25 at 2 mm past the apical foramen with the goal of having the whole canal filled with blood to the level of the cementoenamel junction.
6. Once a blood clot is formed, a premeasured piece of Collaplug (Zimmer Dental Inc, Warsaw, IN) is carefully placed on top of the blood clot to serve as an internal matrix for the placement of approximately 3 mm of white MTA (Dentsply, Tulsa, OK).
7. A (3–4 mm) layer of glass ionomer layer (eg, Fuji IX; GC America, Alsip, IL, or other) is flowed gently over the MTA and light cured for 40 seconds.
8. A bonded reinforced composite resin restoration (eg, Z-100; 3M, St Paul, MN, or other) is placed over the glass ionomer.
9. The case needs to be followed up at 3 months, 6 months, and yearly after that for a total of 4 years.

## Conclusions

Clinicians and researchers have focused for more than 100 years on adequately addressing disinfection to prevent and treat apical periodontitis. Regenerative endodontics also has this primordial focus but also acknowledges principles of bioengineering to promote continued tooth development and normal physiology. Although regenerative endodontic procedures have been highly successful in controlling infection and promoting radiographic root development and nociception (12), recent histologic reports of teeth previously treated with REPs highlight the lack of control over stem cell fate (51, 58). Mineralized deposits along the dentinal walls resemble cementum or osteodentin. In addition, islands of mineralized tissue that resemble bone were found embedded in the loose connective tissue. These findings are in agreement with histologic studies in animal models of regenerative endodontics (59–61) that do not use tissue engineering principles. It is fair to say that clinical success does not appear to match the histologic success (full regeneration resembling a "naive" undamaged pulp). At this time, the significance of these histologic findings to the clinical practice of regenerative endodontics is not clear, but this is the topic of another paper in the symposium. Nonetheless, these findings suggest that the regenerated tissue is not fully recapitulating the native pulp-dentin complex. Significantly more translational research must be performed for all the mechanistic aspects of REPs to reach both clinical and histologic success.

The balance between disinfection and the creation of an intracanal microenvironment conducive for the proliferation of stem cells requires further investigation. Choices of irrigants and medicaments must be made based on their antimicrobial efficacy and with the least harm to stem cells and growth factors present in the microenvironment. Therefore, astute clinicians must make evidence-based decisions on the various chemical and mechanical interventions on stem cells, scaffolds, and growth factors while maintaining the basic principles of disinfection. To date, this requires the interpretation of preclinical studies, and this level of evidence should be increased by randomized controlled clinical studies. Additional translational studies and clinical trials evaluating the different aspects of this procedure are required to understand the complexity of interrelated aspects that could result in better and more predictable outcomes.

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