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Current disinfection protocols in regenerative endodontics -A review

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Abstract

Regenerative endodontics is one of the main emerging branches in endodontics. The branch utilizes stem cells, growth factors and scaffolds to replace cells and tissues that have been damaged or lost due to disease. This potential therapy follows the guidelines released by the European Society of Endodontology and the American Association for Endodontists (AAE) for the treatment of immature permanent teeth (classification of Cvek ,1992)with pulpal necrosis. Regenerative endodontics includes root-canal revascularization, postnatal (adult) stem cell therapy, pulp implant, scaffold implant, three-dimensional cell printing, injectable scaffolds, and gene therapy. Among this, root canal revascularization is more popular in endodontics. This technique receives minimal instrumentation and maximum disinfection with less cytotoxicity to stem cells. This review provides an overview of disinfection protocols in regenerative endodontics and describes possible results in regenerative endodontics. Irrigants and intracanal medicaments play a vital role in root canal disinfection. Use of various concentrations of these disinfectants without affecting the viability of stem cells to provide adequate disinfection is one of the potential challenges in regenerative endodontics. Although AAE recommends 1.5% sodium hypochlorite solution, several studies have shown similar successful results with higher concentrations. Literature search reports the impact of different concentrations of disinfectants on the outcome of revascularisation. By providing an overview of the disinfection protocols required to develop potential regenerative endodontic therapies, we aim to improve these therapies for clinical use.

Keywords: Revascularisation, stem cells viability, chemical disinfection, sodium hypochlorite, laser disinfection

Key Messages: Disinfection or debridement of infected root canal system is an important key for the success of

revascularisation. One of the most challenging aspects of regeneration of pulp-dentin complex is to understand the viability of stem cell with different disinfection procedures for favourable outcome.

Introduction

Regenerative endodontics is defined as "biologically based procedures designed to replace damaged tooth structures, including dentine and root structures, as well as cells of the pulp -dentine complex" [1].This potential therapy primarily focus to save necrotic immature teeth with an open apices.Although Conventional endodontic treatments are generally successful and effective in eliminating pain and controlling infections [2,3].In 1961, Nygaard-Ostby reported the first case of evoked bleeding in the root canal of immature teeth with pulp necrosis[4-5].

Based on American Association of Endodontists (AAE) clinical guidelines[6], success of Regenerative Endodontic therapy greatly depends on the clinical disinfection protocols.

An Overview of Regenerative endodontics

Four goals are there to determine the success of this potential therapy[6].Like any other endodontic treatment Primary goal is to eliminate patient's symptoms and the evidence of bone healing. Secondary goals focused to increase root wall thickness and/or root length and tertiary goals focused to regain tooth vitality. Pinnacle of regenerative goals are the histologic confirmation of dental pulp with an intact odontoblastic layer for structural and functional restoration[7].

Compared to coronal portion of the canal, most bacteria were seen in the apical area and lead to biofilm formation. Author reported on the basis of histobacteriologic studies, lack of mechanical debridement could be the reason for the failure of revascularisation procedure[8].That's why success of revascularisation/regenerative endodontic procedures largely relies on the disinfection of root canal system[8,9]

The American Association of Endodontists suggests a Clinical Considerations for a Regenerative Endodontic Procedure (REP).[6]

Table 1: Regenerative endodontic clinical considerations)[6]

Case Selection	• Tooth with necrotic pulp and an immature apex • Pulp space not needed for post/core, final restoration • No known allergies to antibiotics if intended for use • Compliant patient (parent/guardian)	
Informed Consent	• Two (or more) appointments • Use of antimicrobial(s) • Possible adverse effects: staining of crown/root, lack of response to treatment, pain/infection • Alternatives: MTA apexification, no treatment, extraction (when deemed non-	

	salvageable) • Permission to enter information into AAE database (optional)	
First Appointment	• Local anesthesia, rubber dam isolation, access • Copious, gentle irrigation with 20ml 1.5% NaOCl using an irrigation system that minimizes the possibility of extrusion of irrigants into the periapical space (e.g., needle with closed end and side-vents, or EndoVac). The lower concentrations of NaOCl are advised, to minimize cytotoxicity to stem cells in the apical tissues. • Dry canals • Place antibiotic paste or calcium hydroxide. Ca(OH)2 is antimicrobial at concentrations that do not induce stem cell toxicity and is widely available. As an alternative, if the triple antibiotic paste is used: 1) consider sealing pulp chamber with a dentin bonding agent [to minimize risk of staining] and 2) mix 1:1:1 ciprofloxacin: metronidazole: minocycline in a lower concentration (0.01-0.1 mg/ml) to avoid stem cell toxicity; these lower concentrations appear as a liquid form and are no longer a paste. • Deliver into canal system via Lentulo spiral, MAP system or syringe • If triple antibiotic is used, ensure that it remains below CEJ (minimize crown staining). As an alternative, Ca(OH)2 does not cause staining. • Seal with 3-4mm Cavit, followed by IRM,	• Disinfection Protocols 1. Instrumentation- No or Minimal instrumentation of the root canal system[8,10] 2. CHEMICAL DISINFECTION IRRIGATION- • sodium hypochlorite (NAOCl)[11] • EDTA[12] • chlorhexidine(CHX)[13,14] Intracanal Medicament • Triple antibiotic paste (TAP)[14-19] • Modified TAP[16,18] • TAP conjugated with CHX and calcium Hydroxide[16] • double antibiotic paste (DAP)[20,21] • CHX in association with iodoform[22] • calcium hydroxide isolated or associated with 2.0% of CHX[15,20] 3. Laser Disinfection Single visit regenerative procedure-Photoactivated Disinfection[23,24]

	glass ionomer cement or another temporary material • Dismiss patient for 3-4 weeks	
Second Appointment	• Assess response to initial treatment. If there are signs/symptoms of persistent infection, consider additional treatment with the antimicrobial, or an alternative antimicrobial. Recall the patient in about 3-4 weeks as before. •Anesthesia with 3% mepivacaine without vasoconstrictor, rubber dam, isolation • Copious, slow irrigation with 20ml 17% (Ethylenediamine tetra-acetic acid)EDTA, followed by normal saline, using a similar closed end needle. • Dry with paper points Create bleeding into canal system by over-instrumenting (endo file, endo explorer) • Stop bleeding 3mm from CEJ • Place CollaPlug/Collacote at 3mm below CEJ. • Place 3-4mm of a MTA and reinforced glass ionomer and place permanent restoration. Glass ionomer may be an alternative to MTA in cases where discoloration of the crown is a potential concern.	
Follow-up	• Clinical and Radiographic exam: • No pain or soft tissue swelling (often observed between first and second appointments) • Resolution of apical radiolucency (often observed 6-12 months after treatment) • Increased width of root walls (this is generally observed before apparent increase	

	in root length and often occurs 12-24 months after treatment) • Increased root length • apical closure	

NaOCl is the most commonly used endodontic irrigant in majority of all the regenerative/revascularization reported cases and is now in routine worldwide use[25-27]

6% NaOCl has a negative effect on the stem cell from apical papilla (SCAP) survival[28] and on odontoblastic differentiation of DPSCsinvivo[29,30]. Martin et al. (2014)[30]suggested that this effect can be eliminated with the use of 1.5% NaOCl followed by 17% EDTA. This particular concentration of EDTA completely prevent the profound detrimental effects of the lower concentrations of NaOCl and only partially eliminate the negative effect of 6% NaOCl on the survival of stem cells. Abdel Hafiz Abdel Rahim AS et al concluded that Revascularisation using photo-activated disinfection achieved successful outcomes in the necrotic immature permanent tooth in a single visit[23].

Table 2: Etiology of pulpal disease, Pulpal/periapical diagnosis before REP, Disinfection Protocols, REP clinical outcome and REP scientific outcome of the clinical studies

Publication	Etiology of pulpal disease	Pulpal/periapical diagnosis before REP	Disinfection Protocols	REP clinical outcome	REP scientific outcome
Lui et al (2020)[31]	dens evaginatus	pulp necrosis with chronic apical abscess.	2%sodium hypochlorite, 17% EDTA and intracanal calcium hydroxide (CH)	Successful	Repair
Sabeti et al (2020)[32]	uncomplicated crown fracture	symptomatic irreversible pulpitis with symptomatic apical periodontitis	1.25% sodium hypochlorite,17% EDTA and Calcium hydroxide	Successful	repair
Peng et al (2017)[33]	Dens evaginatus	Previously initiated therapy, symptomatic apical periodontitis	5.25% sodium hypochlorite solution and mixed antibiotics paste of ciprofloxacin, metronidazole, and minocycline	Successful	Combination (repair/ regeneration)
Shimizu et al (2013)[34]	Complicated crown fracture	Pulp necrosis, chronic apical abscess	2.6% sodium hypochlorite and Calcium hydroxide	Successful until fracture	Repair

Jadhav et al.(2013)[35]	Trauma	Pulp necrosis,acute periapical abscess	2.5% sodium hypochlorite,saline and Triple Antibiotic Paste(TAP)	Successful	-
Bezgin et al.(2015)[36]	Caries and Trauma	Pulp necrosis	2.5% NaOCl (20 mL), sterile saline (20 mL),0.12%chlorhexidine (10 mL) , 5% EDTA and triple antibiotic paste	Successful	-
Ray et al(2016)[37]	Trauma	Pulp necrosis with asymptomatic apical periodontitis	0.5% NaOCl and 17% EDTA and double antibiotic paste (DAP), consisting of 200 mg ciprofloxacin and 500 mg metronidazole mixed into propylene glycol.	Successful	-

Literature search reveals the use of NaOCl ,EDTA, calcium hydroxide and TAP in revascularization for disinfection. Sodium hypochlorite (NaOCl) is also widely used as an irrigant in regenerative endodontic procedures Higher concentrations (around 5-6%) of the same has both direct and indirect detrimental effect on survival and differentiation of stem cells[38] and dentin sialophosphoprotein (DSPP) expression[37].In contrast to this , 5.25% of sodium hypochlorite showed successful clinical outcome in regenerative procedures[33].According to the American Association of Endodontics (AAE) sodium hypochlorite should be used at low concentration (1.5%) ,which promote low cytotoxicity to stem cells from apical papilla, maintaining its disinfection ability[30].Moreover, higher NaOCl concentrations have also shown a negative effect on dentin elasticity and flexural strength of thin root walls[39]

Ethylenediaminetetraacetic acid (EDTA-17%) enhances the release of growth factors embedded in the dentin that help in stem cell survival and differentiation[39].Low concentration of NaOCl (0.5% or 1.5%), followed by 17% EDTA as an irrigant counters these deleterious effects on stem cell survival[40,41].

To conclude, disinfection protocol includes appropriate concentration of NaOCl and EDTA as final rinse increase stem cell viability and differentiation.

TAP is the most used intracanal medication in this potential therapy [42].Use of various intra-canal medicaments such as calcium hydroxide,triple antibiotic paste with minocycline (TAP),Double antibiotic paste(DAP) and TAP with amoxicillin, doxycycline, or cefaclor are recommended to ensure disinfection in revascularization[20].Some studies in literature showed fastest discoloration of tooth after TAP placement[43]. Minocycline present in the composition is the cause for tooth staining[44,45].

American Association of Endodontics (AAE) recommended the use of 0.1 mg/ mL to 1 mg/ mL

antibiotic pastes as intracanal medicament to promote stem cell survivability and to maintain chemical disinfection[20,46]Betancourt P et al(2021) suggested the use of Ca(OH)2 over TAP with Doxycycline or other tetracyclines considering the discoloration potential, fluorescence changes and the cytotoxic effect of TAPs for REPS[47].Additionally photo-activated disinfection is a new, selective antimicrobial strategy that can be used successfully in single visit revascularization procedures.[23,24]

Conclusion

Regenerative endodontics is one of the fastest growing branches in endodontics based on biological treatment procedures. Which mainly focused on chemical disinfection rather than mechanical instrumentation? Therefore selection of appropriate chemicals and their ideal concentrations have great role in the success of this therapy. To conclude, Outcome of this treatment primarily depends on the interplay between disinfection of root canal system and stem cell viability.

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