International Journal of Research and Review
Vol. 10; Issue: 10; October 2023
Website: www.ijrrjournal.com
Review Article *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Effects of Irrigants on Pulp Stem Cells: A Systematic Review

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DOI: https://doi.org/10.52403/ijrr.20231060

ABSTRACT

Endodontic intervention in necrotic immature permanent teeth is usually a clinical challenge. With appropriate case selection, regenerative treatment can be effective, providing a desirable outcome. However, there is still no consensus on the optimal methods to achieve predictable clinical outcome. 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 systematic review summarizes the present knowledge regarding the effect of intracanal irrigants on the dental derived stem cells fate in regenerative endodontic procedures.

Keywords: Chlorhexidine; Growth factors; Intracanal irrigants; NaOCl; Regenerative endodontics

INTRODUCTION

Regenerative endodontic procedures (REP) have emerged as an alternative treatment modality for nonessential immature permanent teeth previously treated with apexification. REPs aim to promote normal pulp function by regenerating the pulp-dentin complex for which optimal disinfection of the root canal system is a mandatory call to achieve favorable results in REPs.[1] However, recent regenerative endodontic research indicates that commonly used root canal disinfectants may have direct or indirect effects on stem cells. Several clinical approaches have recently been proposed to preserve and stimulate dental stem cells and mostly focused on chemical disinfection of the root canal system with a combination of sodium hypochlorite (NaOCl) and chlorhexidine (CHX).[2,3] These irrigants are used for their known bacteriocidal and bacteriostatic effects. Unfortunately, there is no standard treatment protocol for the use of different irrigation solutions during disinfection of the root canal. Treatment protocols are implemented without adequate information about the effect of disinfection methods on stem cell viability. These materials can be cytotoxic to stem cells as they have been reported to be cytotoxic to periodontal ligament stem cells, cultured fibroblasts, and human deciduous teeth stem cells.[4] However, in addition to the better known antimicrobial properties, there is a need to evaluate the effect of each chemical agent used in regenerative procedures on stem cell

of apical papilla. The purpose of this review was to comprehensively cover the disinfectants commonly used in regenerative endodontics and their role in stem cell fate.

2. MATERIALS AND METHODS

2.1. Protocol and registration

This work followed the recommendations of the Preferred Reporting Units of Systematic Reviews and Meta-Analyses (PRISMA)[5] statement and was performed according to current recommendations for endodontic systematic reviews and meta-analyses.[6] The systematic review protocol was previously registered in the Prospective Register of Systematic Reviews (PROSPERO) under registration number CRD42021255271.

2.2. Eligibility Criteria

The PICO method (Participant, Intervention, Comparison and Outcome) was used to develop the research question: "What is the effect of different irrigation agents, their concentration, irrigation protocol and irrigation techniques on the survival of the pulp stem cells? ", where (P): patients under 30 years old, traumatized necrotic immature permanent incisors with or without periapical radiolucency, (I): different irrigation methods for pulp revascularization, including irrigants such as sodium hypochlorite in different concentrations, chlorhexidine and EDTA. Another intervention was irrigation techniques used in the treatment of traumatic necrotic immature permanent incisors, (C) Effects of normal saline on pulp stem cells (O): survival, clinical and radiographic success. pulpal revascularization in the treatment of necrotic immature permanent traumatized incisors.

2.3. Outcome measures

The primary outcomes were survival, clinical and radiographic. Survival was defined as a tooth remaining in the oral cavity after follow-up. Clinical success was achieved with absence of clinical sign and symptoms (i.e, tenderness on percussion or palpation, swelling or fistulas, or spontaneous pain). Radiographical success was achieved if the size of the periapical area decreased and side effects such as root resorption and ankylosis were absent or did not increase after follow-up. Secondary outcomes were: sustained root development, pulp viability and crown color, where sustained root development had 3 aspects: increased root length, increased root width and decreased tip diameter.

2.4. Study selection criteria

Inclusion criteria were randomized clinical trials, retrospective/prospective cohort studies and case-control studies. This review included only studies with a follow-up of at least 12 months and studies with at least 10 cases. Only articles that investigated pulpal revascularization techniques (RET) or compared RET with other techniques (i.e, apexification) in traumatized necrotic immature teeth were included. Studies were not restricted by language or year of publication.

2.5. Databases and search strategy

The database search, study selection, and data extraction were performed by two independent researchers (M.S.; I.A.). If there were differences between them, a third author (V.F.L.) was consulted. A systematic expanded electronic search was conducted in PubMed, Web of Science, Scopus, and Embase on June 16, 2021. The search was conducted using the Boolean operators AND and OR to combine terms and develop a search strategy. Search terms were constructed as follows: (regenerative endodontics or regenerative* or endodontic regeneration or regenerative endodontics or regenerative access or pulp revascularization or revascularization* or resuscitation* or loose tips or immature teeth) AND (necrosis) (non-essential AND tooth *) OR pulpless) AND (dental trauma* OR traumatic* OR traumatic* OR tooth In addition, the reference lists of all selected articles were examined for further studies.

2.6. Study selection process

All study titles and abstracts were independently assessed by two reviewers (M.S. and I.A.). If screening of the abstract did not provide sufficient information, the entire article was reviewed before a final decision was made. Two researchers also extracted all data on relevant variables. An investigator not involved in the selection process (J.M.M.C.) performed the following meta-analysis.

2.7. Unpacking the data

The variables extracted from each article were: author and year of publication, study type, sample size, number of groups, demographic variables (sex and age), follow-up in months, loss to follow-up, type of trauma, irrigation agents used and their concentrations, root canal dressing, duration of root canal dressing in weeks, type of frame with or without matrix, type of crown density, survival rate, success rate, failure, changes in root length and width, changes in apical diameter, adverse events or effects and crown and tooth

2.8. Methodological Quality Assessment

Two researchers assessed the risk of bias in all selected studies (M.S. and J.M.M.-C.) using a Cochrane risk-of-bias tool, RoB 2.0, to evaluate randomized clinical trials and the ONS (Newcastle–Ottawa scale) to evaluate non-randomized studies, including case-control and cohort studies. The NOS includes 8 items with a potential score of 9. Three main domains are considered: patient selection, comparability of the study groups, and results or outcomes. Articles were classified as being of 'high', 'moderate', or 'low quality', where high-quality articles scored more than 6 points. The Cochrane RoB 2.0 methodology assessment consists of five domains that evaluate: the randomization process, deviations from intended interventions, missing outcome data, outcome measurement, and selection of reported outcomes. Producing three levels of bias: 'low risk of bias', 'some concerns', or 'high risk of bias'.

2.9. Quantitative Synthesis-Meta-Analysis

All studies included in the meta-analysis were combined using a random-effects model. The estimated effect size was the event rate, odd ratios, means, and different means. The 95% confidence intervals were calculated for all estimated variables. Heterogeneity among the combined studies was assessed using a Q test (p-value < 0.05) and quantified with the I2, considering slight heterogeneity if it was 25–50%, moderate if 50–75%, and high heterogeneity if > 75%. Statistical significance was tested using a Z test (p-value < 0.05). The meta-analysis has been represented with a forest plot, and the publication bias was assessed using an Egger's test that indicates the existence of possible publication bias when the p-value is less than 0.05 (indicating significant asymmetry).

3.1. Study Selection

The search identified a total of 322 initial results, of which 139 were retrieved from PubMed, 50 from Scopus, 85 from Web of Science, 45 from Embase. Duplicates were manually removed using Mendeley reference management software, leaving a total of 199 studies. After screening the title and abstract, 190 articles were excluded. A total of 9 studies were eligible for full-text reading and all were selected for qualitative and quantitative synthesis. (Figure 1)

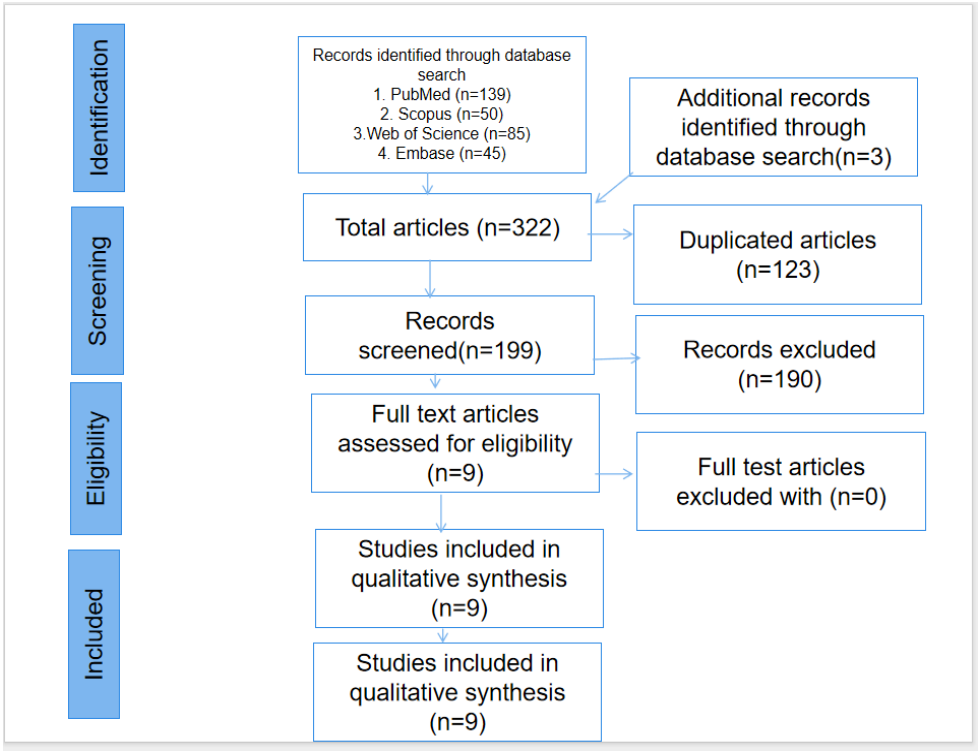

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram.

DISCUSSION

Successful endodontic treatment depends on effective mechanical and chemical cleaning of the root canal system. Mechanical instrumentation of canals infected with immature roots is contraindicated due to fragile, underdeveloped tooth walls. Thus, chemical cleaning is the most important disinfection technique in regenerative endodontic treatment.[7] Previous studies[8,9] have shown that apical papilla stem cells are essential for regenerative endodontics and their preservation should be a priority for clinicians. Martin *et al*[10] reported that different concentrations of NaOCl can affect the survival rate of stem cells and recommended a NaOCl concentration of 1.5-3% during RET. The studies included in this systematic review used different concentrations of NaOCl: 0.5%, 1%, 1.5%, 2%, 2.5%, 5% and 6%; therefore, lower or higher NaOCl concentrations may have negatively affected the RET result.[11-16]

Several studies[17,18] have recommended the use of ethylenediaminetetraacetic acid (EDTA) at the second RET follow-up visit because it reduces the unwanted effects of NaOCl on stem cell viability and releases growth factors. Although another study by Farhad Mollashah *et al*[19] showed that EDTA had greater cytotoxicity than NaOCl and CHX had less cytotoxicity than NaOCl or EDTA on survival of human apical papillary stem cells. In addition, a recent systematic review[20] reported that there were no unsuccessful cases in which EDTA was not used as an irrigation solution during RET procedures. Two of the studies[21,22] included in this review did not use EDTA irrigation and still had similar results to the other included studies. Further studies on the use of EDTA in RET are needed to better understand its effect on such treatments.

EDTA stimulates the release of these growth factors from dentin and increases their bioavailability. In addition, EDTA removes the smear layer and disinfects dentin, thus improving stem cell attachment. Regardless of the effect of EDTA on the release of bioactive molecules, apical extrusion of EDTA not only causes periapical decalcification of bone, but can also impair neuroimmune regulation even at very low concentrations. Also, leakage of EDTA into periapical tissues can inhibit macrophage function and reduce periapical

inflammatory responses. Despite the beneficial use of EDTA in clinical regenerative endodontic therapy, EDTA had direct negative effects on cell proliferation, cell migration and osteogenic differentiation *in vitro* in stem cell of apical papilla.[23,24,25]

In contrast to EDTA, NaOCl inhibited the differentiation of humen deciduous teeth stem cells and pulp stem cells into pre-odontoblast cells *in vitro* and *in vivo studies*.[26] NaOCl denatures dentin-derived growth factors and inhibits their effects on the differentiation and proliferation of mesenchymal stem cells.[27]

Yasuda *et al*[28] showed that the cytotoxicity of MTAD against MC3T3-E1 osteoblast-like cells and periodontal ligament cells was lower than 5.25% NaOCl, 17% EDTA and 0.12% CHX. The cytotoxicity of these materials was evaluated in L929 fibroblasts for 24 h. In the study by Zhang *et al*[29], MTAD showed lower cytotoxicity than 5.25% NaOCl and EDTA and higher cytotoxicity than 2.63%, 1.31% and 0.66% NaOCl. Ring *et al*[30] showed that the cytotoxicity of NaOCl/MTAD was slightly lower than that of NaOCl and NaOCl/EDTA. This indicated higher biocompatibility of MTAD than NaOCl. Another study also confirmed the cytotoxicity of 17% EDTA even at 0.1% dilution. The difference in results was due to the difference in sensitivity of the cell lines used or the experimental conditions, such as the use of different material concentrations and different evaluation times. Conversely, Ghandi *et al*[31] reported that MTAD irrigation had no advantage over saline and did not induce fibroblast adhesion. According to the results of previous studies and the present findings, MTAD should not be chosen as irrigation for revascularization procedures, because it has not been confirmed to have a positive effect on the attachment of stem cells of apical papilla to dentin.

QMix is a mixture of polyaminocarboxylic acid, salt, antimicrobial bisbiguanide, calcium chelating agent and surfactant. It has antimicrobial properties and substance, but cannot dissolve tissue. In several studies, the cytotoxic effect of QMix was found to be very similar to 5.25% NaOCl and greater than 2% CHX.[32,33] In a study by Chandrasekhar *et al*[34], QMix showed less cytotoxicity than 3% NaOCl, 2% CHX, and 17 TA in rat subcutaneous tissues. CHX had the lowest cytotoxicity after sterile saline. The cytotoxicity of CHX, unlike MTAD, EDTA, QMix and NaOCl, did not change significantly over time. In a previous study, 2% CHX showed residual antibacterial activity and was more potent than 5.25% NaOCl in this regard. In addition, it showed lower cytotoxicity compared to 5.25% NaOCl. Bajram *et al*[35] in a study, 2% CHX had greater cytotoxicity to rat periodontal ligament fibroblasts than MTAD and NaOCl. The results of an *in vitro* study on the cytotoxicity of CHX against human gingival cells showed that the toxic power of CHX depends on the composition of the exposure medium, the exposure dose, and the length of exposure. Chlorhexidine does not appear to have long-term toxic effects on host tissues, but it can cause an inflammatory response in these tissues.

CONCLUSION

Chlorhexidine had the lowest cytotoxicity compared to EDTA, MTAD, QMix and NaOCl, and its cytotoxicity did not change over time compared to other solutions. These findings highlight that even commonly used endodontic irrigants have a profound effect on stem cell survival and differentiation capacity. Thus, besides adequate disinfection, it is crucial to create a microenvironment in root canals that will promote the survival/proliferation and differentiation of stem cells. These results can provide a key for choosing the irrigating solution in cases of pulp regeneration.

Declaration by Authors

Ethical Approval: Not Applicable
Acknowledgement: None
Source of Funding: None
Conflict of Interest: The authors declare no conflict of interest.

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How to cite this article: Preeti Mishra, Vineeta Nikhil, Shalya Raj, Priyanka Mishra, Sonal Sahu. Effects of irrigants on pulp stem cells: a systematic review. *International Journal of Research and Review*. 2023; 10(10): 477-483. DOI: *https://doi.org/10.52403/ijrr.20231060*

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