REVIEW ARTICLE

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Viability of human Mesenchymal stem cells from the periradicular region at different concentrations of sodium hypochlorite – systematic review

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Abstract

Pulp revascularization is the procedure that stimulates apical development and root maturation which is essential to secure both survival and viability of stem cells for the success of the regenerative endodontic treatment. The aim of this review is to investigate whether different concentrations of sodium hypochlorite (NaOCl) allow greater viability of human mesenchymal stem cells (MSCs) from the periradicular region, without compromising root canals disinfection. The review is registered in the PROSPERO database under protocol ID CRD42020203863. A search was conducted with no language restrictions in the Medline/PubMed, Scopus, Web of Science databases, as well as a manual research, between March – June 2022, followed by study selection and data extraction according to the PRISMA protocol. The search initially identified 999 articles, of which 883 articles remained after the exclusion of duplicates. Of these, 11 were selected for full reading; 5 of which were considered eligible to compose the qualitative synthesis. Articles that used human mesenchymal stem cells from the apical papilla and periodontal ligament were included. The studies agreed that there is more than 50% loss of cell viability when the culture is in contact with high concentrations (5–6%) of NaOCl after 10 minutes of contact. Low concentrations of NaOCl (0.5–2.5%) provided 80–40% cell viability when in contact for 24 hours. The results of *in vitro* studies assessed in this review indicate that low concentrations of NaOCl allow higher viability of stem cells from the periradicular region, therefore, they are more recommended in cases of pulp revascularization.

Key words

mesenchymal stem cells, regenerative endodontics, sodium hypochlorite

INTRODUCTION

Pulp revascularization is a procedure that stimulates apical development and root maturation. It is well established that infection of the root canal system (RCS) inhibits the regenerative process of the pulp tissue [1]. Additionally, it is essential to secure both survival and viability of stem cells for the success of the regenerative endodontic treatment, since these will be responsible for the proliferation and differentiation among odontogenic epithelial stem cells that will originate the new tissue inside the root canal [2–4].

In this sense, the ideal chlorine-containing endodontic irrigants should demonstrate a balance between antimicrobial efficacy and the ability to create an intracanal micro-environment that favours fixation, proliferation and differentiation of mesenchymal stem cells (MSCs) in this region [5]. Sodium hypochlorite (NaOCl) is the most commonly used irrigating solution for RCS disinfection [6] due to its antimicrobial action and ability to dissolve organic matter [7].

In cases of pulp revascularization, when dental elements have incomplete root development, it is necessary to minimize mechanical preparation of root canals to prevent root weakening. Therefore, it is essential to use auxiliary chemical substances to achieve disinfection of the root canal system [8].

However, researchers are not unanimous on which concentration of NaOCl should be used in cases of endodontic regeneration. It is known that the use of NaOCl in high concentrations (between 5 – 6%) can be a therapeutic manoeuver to increase the disinfection capability during reduced mechanical preparation [9]. At the same time, other scientific publications maintain that the exposure of MSCs to irrigating solutions in high concentrations can suppress the regenerative capacity of MSCs and may prevent the recruitment of new undifferentiated mesenchymal cells, impairing apical repair [5, 9–10].

There is still no consensus and solid scientific knowledge regarding the appropriate therapy for root canal disinfection

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and preservation of mesenchymal cells in the periapical region to achieve success in regenerative endodontic procedures[11]. Thus, this systematic review sought to answer the following research question: What is the effect of different concentrations of NaOCl on MSC mortality from the papilla and periodontal ligament of the periapical region? Therefore, the aim of this study is to investigate whether different concentrations of sodium hypochlorite (NaOCl) allow greater viability of human mesenchymal stem cells (MSCs) from the periradicular region, without compromising root canals disinfection.

MATERIALS AND METHOD

This systematic review was conducted in compliance with the PRISMA guidelines (http://www.prisma-statement.org), and is registered on the International Prospective Registry of Systematic Reviews platform at the University of York, York, United Kingdom (PROSPERO), ID CRD42020203863.

This study aimed to answer the following research question: Do different concentrations of NaOCl exert cytotoxicity on mesenchymal stem cells from the papilla and periodontal ligament of the periapical region? Accordingly, the PICOS model (Patient/Population, Intervention, Comparison and Outcomes) was utilized: **P –** human mesenchymal stem cells from the periradicular region; **I** – use of sodium hypochlorite as an irrigating solution for the root canal system; **C** – control substance−saline/distilled water/culture medium; **O** – viability of human mesenchymal stem cells from the periradicular region; [and **S** – *in vitro* study].

Search strategy. Advanced and systematic searches were carried out with no language restrictions in the PubMed/MEDLINE, Web of Science and Scopus databases, grey literature (OpenGray and REBEC) from March – June 2022, publications were searched without a year limit, the last search was conducted on September 2023, as well as a manual search with key articles selected for this review. The search strategy included four terms from MeSh (Medical Subject Heading)−mesenchymal stem cells, sodium hypochlorite, root canal irrigants, regenerative endodontic; and two uncontrolled descriptors/keywords–apical papilla stem cells and pulp revascularization. The Boolean operators AND and OR were used to incorporate the terms related to the research question. Thus, the database search was conducted as follows: [apical papilla stem cells OR mesenchymal stem cells] AND [sodium hypochlorite OR root canal irrigants] AND [pulp revascularization] OR [regenerative endodontic].

Eligibility criteria. Inclusion criteria were randomized clinical trial *in vitro* studies, with no language restrictions, using human MSCs from the periradicular region (apical, papilla and periodontal ligament), and the use of NaOCl as irrigating solution in its most commonly used concentrations. Studies that did not describe NaOCl concentrations, studies that did not specify exposure time and/or incubation with NaOCl, case series, case reports, literature review and clinical trials, as well as studies that could not be accessed in full, were excluded from the synthesis.

The inclusion and exclusion criteria were established by consensus among all authors considering the research question and the objectives of the study, in an attempt to obtain a wide range of sources to identify as many eligible studies as possible.

Data collection. The search and data extraction were performed by two independent examiners (MCVO and ZBBMF) previously trained and calibrated (kappa = 0.80). Doubts and indecisions were discussed and resolved by consensus, and when necessary, a third reviewer (PRA) was consulted.

Study selection. Potentially eligible studies were inserted into the Mendeley reference manager (Elsevier, Amsterdam, Netherlands) (MCVO) and EndNote reference management software (Clarivate Analytics, Web of Science Group) (ZBBMF) to search and eliminate duplicates. After analyzing the titles and abstracts according to the eligibility criteria, the eligible studies were selected for full reading.

Data synthesis. The following data was collected: types of MSCs (apical papilla and periodontal ligament) from the periradicular region and origin; concentration of sodium hypochlorite-based irrigating solution; exposure time; solutions used as control; and cell viability after exposure to irrigating solutions at different concentrations. If the details were not clear to the reviewers, the authors were requested clarification per e-mail.

Risk of bias assessment. The criteria used to assess the risk of bias in the included studies were established according to the methodology used by Marques et al. [12] for cell culture studies. In these studies, the following items were evaluated: cell type, culture medium, cell culture storage, cell passage number, number of plated cells per well, number of experiment repeats, and description of the methodology for result evaluation. The assessment was performed by two reviewers, who classified the article as 'yes' if the data could be found, or 'no' if the data was not provided. Each article received a score, namely high, moderate or low risk of bias. A score between 6 and 7 positive responses was considered low risk; between 4 and 5 as moderate risk; and between 1 and 3 as high risk of bias.

RESULTS

Selected studies and flowchart. The flowchart describes the search strategy, study selection in the databases, as well as exclusion criteria (Fig. 1).

The major characteristics of the included studies are summarized in Table 1, namely, types of stem cells (most of which are SCAPs of lower 3rd molars), cell culture media and exposure conditions of cell culture to NaOCl, cell passage number, number of experiment repeats, control group, NaOCl concentrations and period of exposure of cells to NaOCl.

Table 2 displays the loss of cell viability found in the studies at high (5–10%) and low concentrations (0.5–3,0%) of NaOCl. The main finding was that lower concentrations of NaOCl increased the viability of the researched stem cells.

Table 3 shows the results of the risk of bias assessment. Two studies were classified as moderate risk [13–14], whereas the studies conducted by Martin et al. [9], Sherestha et al. (15) and

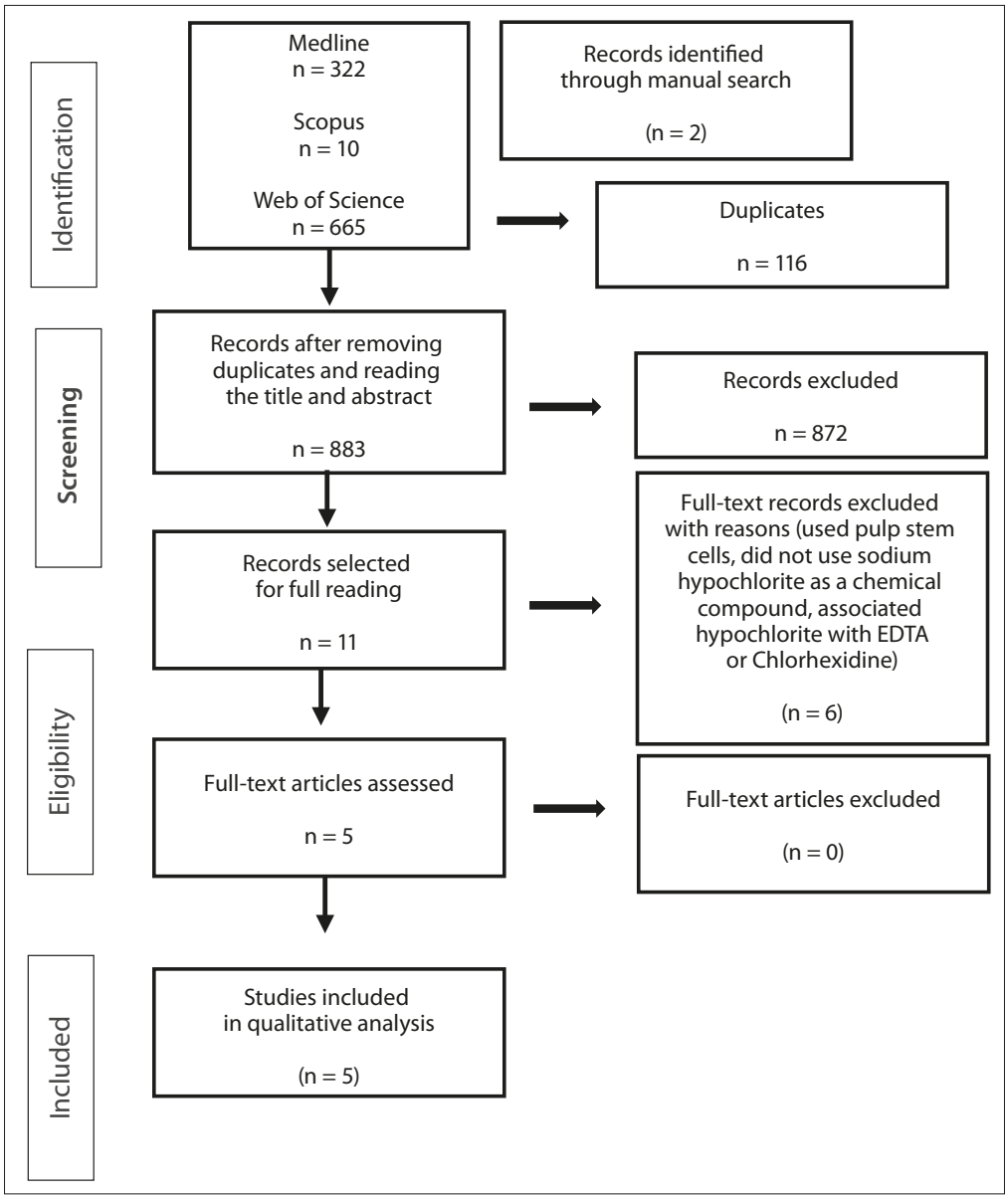

Figure 1. Flowchart including the selection process in the systematic review

Liu et al. [16] were ranked as low risk of bias. The parameter most frequently absent was the number of experiment repeats (42.85%) [13–15].

DISCUSSION

The permanence of viable MSCs within the periradicular tissues is crucial for the success of pulp revascularization therapy [17–19]. In this sense, NaOCl should promote adequate disinfection of RCS without affecting the viability of MSCs from the periradicular region. In this study, we sought to synthesize scientific information about the influence of different concentrations of NaOCl on the viability of MSCs from the periradicular region. The findings allowed us to observe that the cytotoxicity exerted by NaOCl on periapical MSCs depends on the concentration used.

Several methodologies were used to evaluate cell viability of stem cells from the apical papilla (SCAPs) and periodontal ligament stem cells (PDLSCs) exposed to NaOCl in *in vitro* studies [7,9,13–16]. However, regardless of the protocol used (24, 48 or 96 wells per plate for exposure periods of 2, 4, 8, and 24 hours), the results are similar in terms of more than 50% loss of cell viability when the culture is in contact with high concentrations (5.0%-6.0%) of NaOCl after 10 minutes of contact [13–15]. Therefore, seeking to optimize future research in the area, the authors suggest the use of 96-well plate given the minimum volume of culture medium needed for the tests, favouring higher cost-effectiveness thresholds.

The proliferation and differentiation of SCAPs and PDLSCs in the root canal space are responsible for the formation of new tissue, resulting in tissue repair and regeneration [15, 17–19]. Consequently, it is indicated that the irrigating solution remain in contact with the MSCs during an adequate period to promote antimicrobial action, and not impair the differentiation and proliferation of these cells [9].

Thus, strategies to circumvent the use of low concentrations of NaOCl while maintaining adequate RCS disinfection should be employed. Factors such as irrigation volume, association with an ultrasonic irrigation method (active or

Table 1. Characteristics of the included studies

Study	MSC	Cell Culture Storage	Number of Cells	Cell Passage Number	Number of Experiment Repeats	Concen-tration	Exposure Time	Control and Cell/ Solution	Analysis † / ☆
Martin et al. (2014)	SCAPs of lower 3rd molar	NI	24 well plates at 2.5 x 10^5	3rd and 5th	3 times	0.5% 1.5% 3% 6%	Hydrogel scaffolds 5 or 7 days	Hydrogel scaffolds	CellTiter-Glo Luminescent Cell Vitality Assay (Promega, Madison, WI)
Sherestha et al. (2016)	SCAPs of lower 3rd molar	5% CO_2	24 well plates at 1 x 10^5	3rd and 5th	NI	5.25%	Dentine -NaOCl in well plates for 10 min	Not treated in CM	Calcein, AM, cell-permeant dye
Mollashahi et al. (2016)	SCAPs of lower 3rd molar	5%CO_2 95% O_2 37°C-incubation	NI	4th	NI	5.25%	NaOCl in well plates for 1 /5 /15 min	NaCl sterile solution	Cell viability assay (MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide)
Scott et al. (2017)	SCAPs of lower 3rd molar	5% CO_2 37 °C – incubation	NI	NI	NI	6%	NaOCl in well plates for 10'/1h/24h	Distilled water	Calcein, AM, cell-permeant dye
Liu et al. (2018)	PDLSCs /NI	5% CO_2	96 well plates at 1 x 10^5	NI	3 times	0.5% 1,0% 2.5% 5% 10%	NaOCl in well plates for 2 /4/8/24h	Not treated in CM	Cell Viability Assay Kit -Cell Counting Kit-8 (CCK-8) Dojindo; Tokyo, Japan

MSC – mesenchymal stem cells; † / ☆ – cell death/viability; SCAP – Stem Cell from the Apical Papilla; NI – not identified; CM – Culture Medium; PDLSC – periodontal ligament stem cell.

Table 2. Loss of cell viability

Authors	Loss of cell viability	% NaOCl	Loss of cell viability	% NaOCl	Time
Martin et al. (2014)	37%*	0.5% 1,5% 3,0%	85,27%*	6%	7 days
Sherestha et al. (2016)	-	-	50%**	5.25%	10 min
Mollashahi et al. (2016)	-	-	58%*	5.25%	15 min
Scott et al. (2017)	-	-	80%* 100%* 100%	6% 6% 6%	10 min 1h 24h
Liu et al. (2018)	20%*	1,0%	-	-	2h
Liu et al. (2018)	40%*	1,0%	-	-	4h
Liu et al. (2018)	60%*	1,0%	-	-	8h
Liu et al. (2018)	70%*	1,0%	-	-	24h
Liu et al. (2018)	20%* 30%* 60%*	0.5% 1,0% 2.5%	70%* 80%*	5% 10%	24 h

* Loss of cell viability compared to total cells in the group conditioned with NaOCl; ** Loss of cell viability of cells conditioned with NaOCl, compared with cells conditioned with the bioactive molecule releasing nanoparticle system.

passive) or photodynamic therapy, and frequent changes, may be alternatives to increase its anti-microbial action [20–23]. Moreover, retention time of NaOCl in the RCS can contribute to a deeper penetration of the solution in the different ramifications, increasing the chances of the irrigant to reduce bacteria counts in these places [24].

Although *ex vivo* research has shown that NaOCl concentration is not the only variable to be considered during RCS disinfection, it was observed that the higher the NaOCl concentrations, the greater the anti-bacterial efficacy [14, 25]. However, the greater cytotoxicity of NaOCl at high concentrations was due to the high pH of the substance, which releases hydroxyl ions and alters the integrity of the cytoplasmic membrane of SCAPs and PDLSCs, causing irreversible chemical injuries to organic components and the nutrient transport system [26]. Furthermore, the generated cytotoxicity activates the function of phagocytes which initiates a local inflammatory process [27].

Hence, the authors of this study underscore the significance of employing lower concentrations of NaOCl to enhance the likelihood of a successful pulp revascularization process. This aligns with a previous study [16] which revealed that when MSCs are exposed to lower concentrations of NaOCl for a short time interval, higher cell viability occurs. Furthermore, the literature suggests the association of low concentrations of NaOCl with 17% EDTA [28]. EDTA could be effective in removing the smear layer, cleaning the root canal surface from microorganisms, allowing the environment to become conducive to cell proliferation, and promoting migration, adhesion and differentiation of stem cells from the periradicular region [29–31].

Table 3. Methodological quality and risk of bias assessment of included studies

Study	MSC	CM	Cell Passage Number	Cell Culture Storage	Number of Cells	Number of experiment repeats	Methods and results	Risk of Bias
Martin et al. (2014)	Yes	yes	Yes	no	yes	yes	yes	Low
Sherestha et al. (2016)	Yes	yes	Yes	yes	yes	no	yes	Low
Mollashahi et al. (2016)	Yes	yes	Yes	no	no	no	yes	Moderate
Scott II et al. (2017)	Yes	yes	No	yes	no	no	yes	Moderate
Liu et al. (2018)	Yes	yes	no	yes	yes	yes	yes	Low

MSC: mesenchymal stem cells; CM: Culture Medium. Adapted from Martin et al. (9)

The findings of this study have to be seen in the light of some limitations, namely, the scarcity of *in vitro* studies; the dearth of standardization of some important data, such as exposure time of MSCs to NaOCl; and the paucity of information on cell passage number, wells and culture medium. Therefore, we propose that further *in vitro* studies should be conducted to standardize the methodology, so that future pre-clinical studies and irrigation protocols in regenerative endodontic procedures can be developed based on the results.

CONCLUSION

According to the results presented in this review, a lower concentration of irrigating solutions maintained higher vitality of stem cells from the periradicular region and, therefore, they are more recommended in cases of pulp revascularization.

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