

Assessing the Cytotoxic Effects of Calcium Hydroxide and Triple Antibiotic Paste on SCAP: A Systematic Review

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ABSTRACT

Aim: This systematic review paper focuses on the effects of two common intracanal medicaments used in regenerative endodontic procedures on the survival of human apical papilla cells.

Background: Periapical lesions form due to an inflammatory response triggered by microorganisms and their by-products invading the root canal system. Endodontic therapy aims to eliminate these microbes and disinfect the root canal system. Reducing the microbial load is crucial before obturation, as it helps alleviate symptoms and reduces the incidence of persistent periapical pathosis. This study aims to evaluate and compare the effects of calcium hydroxide and triple antibiotic paste (TAP) intracanal medicaments on the survival of human apical papilla cells.

Materials & Methods: An electronic search of the Medline database (via PubMed) and Web of Science was conducted using terms such as calcium hydroxide, TAP, and intracanal medicaments. The search criteria followed the PRISMA statement guidelines. For each selected study, data were extracted: Study design and methodology, Sample size and characteristics, and Type and concentration of intracanal medicament used.

Results: Twenty articles met the established criteria and were included.

INTRODUCTION

The first documented antibiotic use in endodontics was a paste called PBSC & it was reported by Grossman [1]. PBSC was a mixture of Penicillin, Bacitracin, Streptomycin &, and Caprylate Sodium [2]. Penicillin was effective against gram-positive, streptomycin against gram-negative, bacitracin against gram-positive strains & caprylate sodium against yeast. The

combination of Metronidazole & ciprofloxacin decreases one-day-old bacterial biofilm [3].

Periapical lesions are abnormal growth or infections that can occur around the root tip of a tooth. Bacterial infection of dental pulp may lead to periapical lesions. It develops as a sequel to pulpal diseases. The ultimate goal of endodontic treatment is to remove the bacteria from an infected root canal. Antibiotics are suitable materials for using intracanal medicaments. Triple

Antibiotic Paste (TAP) containing Metronidazole, ciprofloxacin, and Minocycline has been used as an intracanal medicament due to its antimicrobial properties [4].

METRONIDAZOLE:

It is a nitroimidazole with a broad spectrum effective against anaerobic bacteria. It destroys bacterial cells by permeating their membrane and binding to DNA, disrupting their helical structure and causing rapid death [5].

CIPROFLOXACIN:

It is a second-generation fluoroquinolone antibiotic effective against *Escherichia coli*, *Klebsiella*, *Enterococcus*, *Streptococcus*, etc. [6].

TETRACYCLINES:

Bacteriostatic inhibit protein synthesis by binding to bacterial ribosomes involved in translation.

TAP [7].

Triple antibiotic paste, commonly used in dentistry and wound care, typically contains a combination of ciprofloxacin, metronidazole, clindamycin, or similar antibiotics. Because it is a combination of existing antibiotics, this formulation is not associated with a specific "discovery" date in the way a new drug might be [8].

Combining antibiotics to prevent or treat infections has been practised for many decades. Still, the specific combination used in the triple antibiotic paste for dental applications, particularly in endodontics (root canal therapy), became popular in the late 20th century [9]. The exact date of the first use of this combination is not well-documented, as it evolved from clinical practices and studies over time rather than from a single discovery event [10].

The survival and proliferation of stem cells from the apical papilla (SCAP) are critical for the success of regenerative endodontic procedures. Intracanal medicaments, such as calcium hydroxide ($\text{Ca}(\text{OH})_2$) and triple antibiotic paste (TAP), are commonly used to disinfect the root canal system and create a conducive environment for tissue regeneration. However, their effects on SCAP viability vary significantly [11].

Calcium hydroxide has long been favoured for its antimicrobial properties and ability to promote complex tissue formation. It creates an alkaline environment that is hostile to most bacteria, encouraging stem cell differentiation and proliferation. Conversely, TAP, a combination of ciprofloxacin, metronidazole, and minocycline, is highly effective against a broad spectrum of endodontic pathogens. Despite its antimicrobial efficacy, concerns about its cytotoxic effects on SCAP have been raised, particularly at higher concentrations [12].

The cytotoxic effects of TAP on SCAP, especially at higher concentrations, have raised concerns about its suitability for regenerative procedures. The potential for TAP to adversely affect stem cell viability necessitates a careful evaluation of its use in clinical practice.

This systematic review aims to critically assess and compare the effects of $\text{Ca}(\text{OH})_2$ and TAP on the survival of human apical stem cells. By synthesising current research findings, this review seeks to provide a comprehensive understanding of how these intracanal medicaments influence SCAP viability.

HUMAN APICAL PAPILLA STEM CELLS (SCAP)

The apical papilla is essentially the apical part of the dental papilla. Sonoyama et al. (2006) collected and cultured cells from the human apical papilla, which was taken from the root end of a young human third molar. They reported that the cells they isolated exhibited mesenchymal stem cell properties. They called those cells stem cells from apical papilla (SCAP) [13].

Stem cells from the apical papilla (SCAPs) are a unique type of dental stem cell found in the apical papilla, which is the tissue at the tip of the root of developing teeth.

Characteristics of stem cells :

- **Origin:** SCAPs are derived from the apical papilla of immature permanent teeth.
- **Type:** They are mesenchymal stem cells (MSCs), which can differentiate into various cell types.
- **Proliferation:** SCAPs are highly proliferative, meaning they can multiply rapidly.

- **Differentiation:** They can differentiate into odontoblasts (cells that form dentin) and other cell types, such as osteoblasts (bone-forming cells) and adipocytes (fat cells) [14-16]

SCAPs has applications in dentistry & regenerative medicines. They are:

- 1) Root development & regeneration: Helps in root maturation in immature permanent teeth & can be differentiated into odontoblast-like cells, helping dentin formation. Helps in regenerative endodontics procedures for the treatment of necrotic pulp, which promotes regeneration of pulp dentin complex
- 2) Dentin Regeneration
- 3) Vascular Engineering
- 4) Contribute to regeneration of periodontal ligament, cementum & alveolar bone

METHOD OF PREPARATION HUMAN APICAL PAPILLA STEM CELLS

The apical papilla tissue was excised from the dental pulp with a sterile scalpel. Stem cells were extracted from the apical papilla using enzymatic digestion utilising 2 mg/ml of type I collagenase in Dulbecco's modified Eagle's media (DMEM). The cells were passed in a culture medium with 15% fetal bovine serum. Following four passes, sufficient cell confluence was attained. This process was performed 5 to 8 times to acquire a substantial population of cells. The culture media was renewed every 2 to 3 days, and the cells were subcultured after 1 week [17,18].

Flow cytometry was conducted to verify the mesenchymal origin of the cultivated cells by assessing the percentage of mesenchymal cells through the identification of CD90 and CD105 mesenchymal markers (Chemicon, USA) and the CD45 hematopoietic marker (Chemicon, USA) [19].

Removing the cells stuck to the bottom of the flask was necessary to freeze the cells. The overlying media was removed to achieve this, and the flask holding the cells was subsequently rinsed with PBS. The PBS was thereafter discarded, and the flask was subjected to trypsinisation. Following cell separation, a complete culture media was introduced to the flask. The contents were transferred into sterile Falcon tubes and centrifuged (Farxane Arman, Iran) at 800 rpm for 7 minutes. The supernatant was removed, and 2 ml of complete culture media was added to the cells settled at the bottom. The cells were subsequently enumerated, and 1 ml of growth media was added every $1-2 \times 10^6$ cells for cryopreservation. The cell suspension was subsequently placed into sterile cryo-tubes and frozen at -20°C for 24 hours. Subsequently, they were relocated to another freezer at -70°C and thereafter to a nitrogen tank. The cryo-tubes were incubated for defrosting, and their contents were transferred to sterile falcon tubes. Subsequently, 3 mL of DMEM was added, and the tubes were centrifuged at 1500 rpm for 3 minutes. The supernatant was removed, and 1 mL of new DMEM with fetal bovine serum was added and incubated [20-25].

Cytotoxicity evaluation: The methyl thiazolyl tetrazolium (MTT) assay: This colourimetric assay quantifies cellular metabolic activity to measure cell viability, proliferation, and cytotoxicity. Mitochondrial dehydrogenase reduces MTT in live cells, forming a purple formazan product [26].

Alamar Blue Assay: It employs resazurin, a non-toxic dye converted to resorufin by living cells, generating a fluorescent signal. It is utilised to evaluate cell viability and cytotoxicity [27]. The LDH assay measures the release of lactate dehydrogenase (LDH) into the culture media due to cell membrane injury. It quantifies the released LDH, correlating with the quantity of injured or deceased cells [28].

Flow Cytometry: This method can assess cell death by labelling cells with annexin V and propidium iodide (PI). Annexin V attaches to phosphatidylserine on the outer leaflet of the plasma membrane in apoptotic cells, whereas propidium iodide (PI) stains the DNA of necrotic cells [29].

The Comet Assay, or single-cell gel electrophoresis, identifies DNA damage at the individual cell level. It is employed to evaluate genotoxicity and cytotoxicity [30].

Scratch-Wound Healing Assay: This assay assesses cell migration and proliferation by inducing a "scratch" in a cell monolayer and monitoring the subsequent closure of the wound over time.5.

Real-Time PCR and Assay: These techniques examine cellular differentiation and proliferation. Real-time PCR measures gene expression levels.

METHODOLOGY

Search Strategy

A complete literature search was done using PubMed, Google Scholar, and Web of Science electronic databases. The search

Search Strategy Table

technique includes keywords and Medical Subject Headings (MeSH) phrases relating to the review issue, such as "Triple Antibiotic Paste," "Calcium Hydroxide," "regenerative endodontics," "diagnosis," and "outcomes." Boolean operators (AND, OR) were employed. A review on triple antibiotic paste as a suitable material for regenerative endodontics combines search phrases and narrows the results.

Element	Details
Databases Searched	- PubMed (via Medline) - Web of Science - Google Scholar
Search Keywords	- Calcium Hydroxide - Triple Antibiotic Paste (TAP) - Intracanal medicaments - Regenerative endodontics - Human apical papilla cells (SCAP) - Cytotoxicity
MeSH Terms (if applicable)	- "Calcium Hydroxide" - "Triple Antibiotic Paste" - "Regenerative Endodontics" - "Human Apical Papilla Cells" - "Stem Cells"
Boolean Operators	- AND (to combine main concepts) - OR (to combine synonyms/related terms) - Parentheses for grouping (e.g., Calcium Hydroxide OR TAP)
Inclusion Criteria	- Studies on regenerative endodontics using SCAP (stem cells from apical papilla) - Use of Calcium Hydroxide or standard TAP in intracanal medicament application
Exclusion Criteria	- Modified TAP formulations - Double antibiotic paste (DAP) - Calcium Hydroxide sealers - Studies on pulp capping - Non-SCAP stem cells - Case reports
Time Frame	No restrictions on the publication date are specified, but studies must be peer-reviewed and in English.
Study Design	- Randomized controlled trials (RCTs) - Observational studies - In vitro and animal studies - Exclusion of case reports and studies with non-human subjects
Key Data Extraction Variables	- Study design - Sample size - Study methodology - Concentration of Calcium Hydroxide or TAP used - Effects on SCAP survival - Cytotoxicity assessment methods

Primary Outcome	SCAP survival rate (measured via cytotoxicity assays such as MTT, Alamar Blue, LDH, etc.)
Secondary Outcomes	- Stem cell proliferation and differentiation - Clinical outcomes such as apical closure, root lengthening, and dentin wall thickening
Bias Assessment	- Evaluating risk of bias using the Cochrane risk of bias tool (for RCTs) - Newcastle-Ottawa Scale for observational studies - Levels: low, moderate, high
Data Synthesis Method	- Qualitative synthesis of findings - Meta-analysis (where applicable)
Search Strategy - PubMed	("Calcium Hydroxide"[MeSH Terms] OR "Triple Antibiotic Paste"[MeSH Terms] OR TAP) AND ("Regenerative Endodontics"[MeSH Terms] OR SCAP OR "Human Apical Papilla Cells") AND (Cytotoxicity OR survival)
Search Strategy - Web of Science and Scopus	("Calcium Hydroxide" OR "Triple Antibiotic Paste" OR TAP) AND ("Regenerative Endodontics" OR SCAP OR "Human Apical Papilla Cells") AND (Cytotoxicity OR survival)

Cochrane Search Strategy

Step-by-Step Search Strategy

Regenerative Endodontics and SCAP

Keywords:

"Regenerative Endodontics" OR "Regenerative Root Canal Treatment" OR "Stem Cell-based Endodontics" OR "Pulp Regeneration" OR "Root Canal Regeneration"

Stem Cells and SCAP:

"Stem Cells from Apical Papilla" OR "Human Apical Papilla Cells" OR SCAP OR "Dental Pulp Stem Cells"

Intracanal Medicaments

Keywords for Medicaments:

"Calcium Hydroxide" OR "Triple Antibiotic Paste" OR TAP OR "Intracanal Medicament" OR "Antimicrobial Medicament" OR "Disinfectant Paste" OR "Endodontic Irrigants"

Cytotoxicity and Viability

Cytotoxicity and Viability Keywords:

Cytotoxicity OR Toxicity OR "Cell Viability" OR "Cell Survival" OR "Cell Proliferation" OR "Stem Cell Proliferation" OR "Cellular Differentiation" OR Apoptosis OR "Cell Death"

Combine Using Boolean Operators

((("Regenerative Endodontics" OR "Regenerative Root Canal Treatment" OR "Stem Cell-based Endodontics" OR "Pulp Regeneration" OR "Root Canal Regeneration")

AND

("Stem Cells from Apical Papilla" OR "Human Apical Papilla Cells" OR SCAP OR "Dental Pulp Stem Cells"))

AND

("Calcium Hydroxide" OR "Triple Antibiotic Paste" OR TAP OR "Intracanal Medicament" OR "Antimicrobial Medicament" OR "Disinfectant Paste" OR "Endodontic Irrigants")

AND

(Cytotoxicity OR Toxicity OR "Cell Viability" OR "Cell Survival" OR "Cell Proliferation" OR "Stem Cell Proliferation" OR "Cellular Differentiation" OR Apoptosis OR "Cell Death")

Bias Assessment

The bias assessment was conducted by systematically evaluating various studies based on three key criteria: bias due to intervention differences, bias in measurement technique, and bias due to duration and exposure. Each study was reviewed for specific indicators of risk associated with these criteria. Researchers assigned a low, moderate, or high-risk level based on the clarity and completeness of the information provided in the studies. For instance, they assessed whether concentrations and durations were specified in the interventions, which is critical for determining potential biases. The findings were categorized according to these risk levels, highlighting studies with missing data or those that relied on existing literature without direct intervention testing. This structured approach enabled a comparative analysis of the bias present in the different studies, facilitating a clearer understanding of the quality and reliability of the research outcomes.

[Figure 1]

Author	Bias due to Intervention Differences	Bias in Measurement Technique	Bias due to Duration and Exposure
Ruparel NB et al. [12]	●	●	●
Pai S et al. [19]	●	●	●
Ehrmann EH [20]	●	●	●
Metzler RS et al. [21]	●	●	●
Jamshidi D et al. [22]	●	●	●
Hoshino E et al. [23]	●	●	●
Kitikuson P et al. [24]	●	●	●
Kontakiotis EG et al. [25]	●	●	●
Kim SG et al. [26]	●	●	N/A
Berman LH et al. [27]	●	●	N/A
Benz V et al. [28]	●	●	N/A
Murad M et al. [29]	●	●	●
Vishwanat L et al. [30]	●	●	●
Hutton B et al. [31]	●	●	N/A
Deeks JJ et al. [32]	●	●	N/A
Jain G et al. [33]	●	●	●
Moranker R et al. [34]	●	●	●
Jagdale S et al. [35]	●	●	●

Figure 1: Risk of Bias Assessment

Green - Low Risk

Yellow - Moderate Risk

Red - High Risk

Study Design

This systematic review was designed to evaluate the efficacy and impact of regenerative endodontic procedures involving human apical papilla cells and SCAP (stem cells from the apical papilla), specifically utilizing calcium hydroxide and Triple Antibiotic Paste (TAP). A comprehensive literature search across multiple databases identified relevant studies focused on human subjects and limited to peer-reviewed articles published in English. Screening was conducted according to pre-defined inclusion and exclusion criteria, emphasizing studies on SCAP and excluding those involving modified TAP, double antibiotic paste, pulp capping, calcium hydroxide-based sealers, other stem cells, and case reports, to ensure a targeted focus on the most relevant

interventions. Data extraction employed a standardized template to record essential details, including author names, publication year, study design, sample size, key findings, methodological quality, and limitations, ensuring consistent handling across all studies. A qualitative synthesis provided an overall assessment, supplemented by meta-analysis where data allowed, to quantitatively summarize findings. Each study's quality was assessed using the Cochrane Risk of Bias tool for randomized controlled trials and the Newcastle-Ottawa Scale for observational studies. Although no new ethical approval was required, the review adhered to ethical standards to ensure research integrity and transparent reporting. This approach enabled a thorough review of SCAP-based regenerative procedures in endodontics, clarifying their effectiveness and potential impact in the field.

Inclusion and Exclusion Criteria

Inclusion Criteria:

The inclusion criteria for the study focus on specific regenerative endodontic procedures, particularly involving human apical papilla cells and SCAP (stem cells from the apical papilla), as well as the use of calcium hydroxide and Triple Antibiotic Paste.

Exclusion Criteria:

Conversely, the exclusion criteria eliminate studies involving calcium hydroxide-based sealers, pulp capping, modified Triple Antibiotic Paste (TAP), double antibiotic paste (DAP), stem cells other than SCAP, and case reports. This selection ensures a targeted and specific scope for the study, focusing on the most relevant and impactful factors related to regenerative endodontics.

Research Questionnaire

1. What is the patient's condition or problem in each study?
2. Does the study focus on using calcium hydroxide and standard Triple Antibiotic Paste for treatment?
3. Are modified TAP, double antibiotic paste, pulp capping, or other stem cells (non-SCAP) excluded in the study?

4. What are the primary outcomes related to the effectiveness and impact of the procedure?
5. How do the results reflect the benefits and limitations of using SCAP in regenerative endodontics?

PICO Format of the Inclusion and Exclusion Criteria :

- **P (Patient/Problem):** Patients receiving regenerative endodontic treatment with SCAP (stem cells from the apical papilla).
- **I (Intervention):** Use of calcium hydroxide and standard Triple Antibiotic Paste.
- **C (Comparison):** Exclusion of studies using modified TAP, double antibiotic paste, calcium hydroxide sealers, pulp capping, or stem cells other than SCAP.
- **O (Outcome):** Assessment of the effectiveness and impact of SCAP-based regenerative procedures with calcium hydroxide and standard TAP.

Category	Details
P (Patient/Problem)	Patients receiving regenerative endodontic treatment with SCAP (stem cells from the apical papilla).
I (Intervention)	Use of calcium hydroxide and standard Triple Antibiotic Paste.
C (Comparison)	Exclusion of studies using modified TAP, double antibiotic paste, calcium hydroxide sealers, pulp capping, or stem cells other than SCAP.
O (Outcome)	Assessment of the effectiveness and impact of SCAP-based regenerative procedures with calcium hydroxide and standard TAP.

Data Extraction and Synthesis

Data Extraction: Data from pertinent research were obtained utilizing a standardized data extraction template, assuring uniformity and comprehensiveness. Subsequent information was documented for each study: the authors and publication year, the study design and sample size, the principal findings and conclusions, and the methodological quality and limitations. This method facilitated a thorough and methodical examination of the existing literature.

Summary:

The retrieved data were qualitatively synthesized to deliver a comprehensive evaluation of the available information regarding the review topic. Where applicable, a meta-analysis was conducted to quantitatively synthesize the results.

Evaluation of Quality

The publications included in the analysis were assessed for quality using established criteria, including the Cochrane risk of bias tool for randomized controlled trials and the Newcastle-Ottawa Scale for observational research. These evaluations determined the quality of each study as high, medium, or low.

Constraints

The review is constrained by limitations, such as possible publication bias and the inconsistency in study designs and results. These considerations were taken into account when analyzing the results and formulating conclusions.

Ethical Considerations: This review of previously published studies did not require ethical approval. It was completed in compliance with the ethical principles of research integrity and reporting.

PRISMA Flowchart

PRISMA Flowchart illustrating the study selection process (Figure 2).

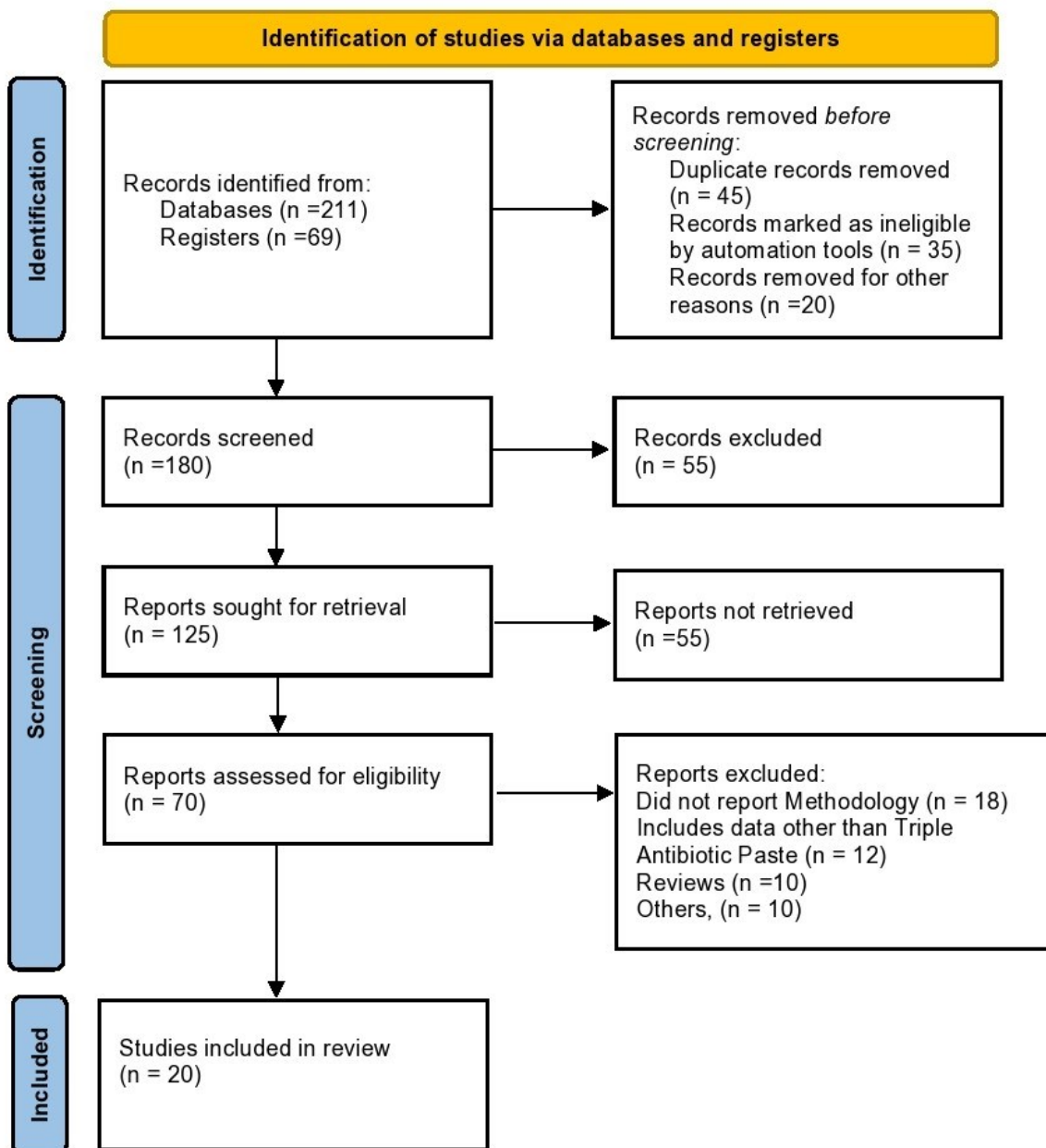


Figure 2: PRISMA FLOWCHART of the Study

DISCUSSION

The objective of this systematic review was to evaluate and compare the effect of calcium hydroxide and triple antibiotic pastes of intracanal medicaments on the survival of human apical papilla stem cells. The results revealed that applying calcium hydroxide has no detrimental effect on human apical papilla stem cells' survival rate at any concentration, whereas triple antibiotic paste at concentrations 1,10, and 100mg/ml resulted in an increase in SCAP survival rate, respectively. Combining these three antibiotics overcomes bacterial resistance and achieves higher antimicrobial action.

American Academy of Endodontic Guidelines 2016 recommended using calcium hydroxide or antibiotic pastes for 1-4 weeks for regenerative endodontic procedures.

Risk of bias assessment is an integral component of any systematic review. It helps to explore the quality of evidence in the literature and draw conclusions from its results.

CALCIUM HYDROXIDE VERSUS TRIPLE ANTIBIOTIC PASTE (TAP) ON HUMAN APICAL PAPILLA STEM CELL(SCAP) SURVIVAL :

Zafar C Cebrelli et al. (2011) conducted a case study on Regenerative Endodontic Treatment (Revascularization) of immature necrotic molars treated with calcium hydroxide. Based on 10 months of clinical and radiographic data, he determined that regenerative endodontic treatment for multirooted, immature, necrotic teeth is a viable alternative to standard apexification treatment. Comprehensive irrigation with 2.5% NaOCl and a three-week insertion of calcium hydroxide in the coronal third of the root canal ensured sufficient disinfection without adversely impacting induced root formation [11]. Ruparel NB et al. (2012) conducted a study on the direct effect of intracanal medicaments on the viability of stem cells from the apical papilla. He determined that elevated concentrations of antibiotics adversely affect the stem cells of the apical papilla

(SCAP), while lower concentrations, such as calcium hydroxide, promote SCAP survival and proliferation [12]. Swathi Pai et al. (2014) conducted an in vivo study on the impact of calcium hydroxide and Triple Antibiotic Paste (TAP) as intracanal medicaments on inter-appointment flare-ups in diabetic patients. He determined both methods are efficacious for treating interappointment flare-ups in diabetic patients. TAP is superior to calcium hydroxide in mitigating flare-ups in diabetic individuals [13]. L. Krishna Prasad et al. (2015) conducted a comparative study on calcium hydroxide and TAP as intracanal medicaments for emergency pain alleviation. He determined that TAP is superior to calcium hydroxide in mitigating the recurrence of discomfort. The total incidence of the mean pain range associated with calcium hydroxide is much greater than that of TAP [14]. Dimitrios G. et al. (2019) conducted an in vitro study on the quantity of stem cells in root canal dentin after applying TAP or calcium hydroxide. He partitioned three groups. Group A: Root canals unfilled. Group B Root canals utilizing Triple Antibiotic Paste (TAP). Group C Root canal treatment utilizing Calcium Hydroxide. After one week, the intracanal medicament was withdrawn, and stem cells were inoculated onto the treated surface of the specimens. Group B or C had a statistically significant increase in cell count [15]. Mohammed Alasgah et al. (2020) conducted a two-year follow-up on the regenerative endodontic therapy of an immature molar with calcium hydroxide and TAP. He determined that a multirooted tooth exhibiting necrotic pulp and periapical radiolucency was effectively treated with a Restorative Endodontic Procedure (REP), as demonstrated clinically and radiographically, utilizing non-setting calcium hydroxide and TAP in succession to ensure thorough canal disinfection [16]. Davond Jamshedi et al. (2021) conducted a study on the cytotoxicity and genotoxicity of calcium hydroxide

and two antibiotic pastes on human stem cells from apical papilla (SCAP). The findings of his investigation indicated that all measured concentrations of calcium hydroxide exerted no influence on stem cells at any period. TAP exhibited cytotoxicity at concentrations of 10 and 100 mg/ml at all periods within 72 hours for 1, 10, and 1000 mg/ml. He ultimately determined that, in contrast to calcium hydroxide, TAP, particularly at elevated concentrations, elicited cytotoxicity and genotoxicity in human SCAP [17].

Viviana Benz et al. (2022) conducted a meta-analysis on the outcomes of restorative endodontics utilizing antibiotic pastes and calcium hydroxide. He determined dentin wall thickening occurred in 66% of cases treated with antibiotic paste, whereas it was detected in 53% of cases utilizing calcium hydroxide. He also determined that applying antibiotic paste resulted in apical closure in 66% of regenerative endodontic treatments. Calcium hydroxide was found to achieve apical closure in 88% of cases. Root lengthening and apical healing were deemed satisfactory for both disinfection regimens [18]. The study conducted by Pai, Vivekananda Pai, Thomas, and Bhat, published in the Journal of Conservative Dentistry in May 2014, examined the impact of calcium hydroxide and triple antibiotic paste as intracanal medicaments on the occurrence of inter-appointment flare-ups in diabetic patients. This in vivo investigation aimed to ascertain whether these frequently utilized drugs affected the incidence of flare-ups between dental treatment sessions. The study assessed the frequency of flare-ups and evaluated the effects of the two drugs, revealing the comparative efficacy of each treatment in treating flare-ups within this specific patient population. The authors comprehensively examined the outcomes, considering the particular circumstances of diabetic patients [19].

AUTHOR	TYPE & CONCENTRATION OF INTRA CANAL MEDICAMENT	MEASUREMENT TECHNIQUES OF CELLS SURVIVAL OR TOXICITY	DURATION & EXPOSURE TO MEDICAMENT	KEY FINDINGS AND STATISTICAL DIFFERENCE
Ruparel NB et al. [12]	TAP(metronidazole,c iprofloxacin,minocyc line) 0.01mg/ml Calcium hydroxide(ultra cal) 1mg/ml	Human extracted molars	SCAP exposed to different drugs at the concentration tested was incubated & 5%CO2for 3 days	Calcium hydroxide significantly increases the proliferation /survival of SCAPat the concentration of 1mg/ml, resulting in an increase of 68.3+_12%
Pai S et al. [19]	Calcium Hydroxide and Triple Antibiotic Paste (Concentration not specified)	Incidence of inter-appointment flare-up in diabetic patients (in vivo study)	Duration exposure and not specified.	The study found that both calcium hydroxide and triple antibiotic paste are effective in managing inter-appointment flare-ups in diabetic patients, and it provided a detailed comparison of their efficacy.
Ehrmann EH [20]	Various intracanal medicaments (specific types and concentrations not detailed)	Postoperative pain assessment in endodontic patients	Postoperative pain measured over time following medicament application	The study examined the relationship between intracanal medicaments and postoperative pain in endodontics, finding that certain medicaments were associated with reduced pain while others had no significant impact.
Metzler RS et al. [21]	Calcium Hydroxide and Triple Antibiotic Paste (Concentration not specific)	Debridement effectiveness in human mandibular molars	Duration not specified	The study found that ultrasonics combined with calcium hydroxide enhanced the debridement process in human mandibular molars.

Jamshidi D et al. [22]	Ciprofloxacin, Metronidazole, MinocyclinCalcium Hydroxide and TAP	Cytotoxicity and Genotoxicity on Human Stem Cells of the Apical Papilla	Duration not specified	The study evaluated the cytotoxic and genotoxic effects of calcium hydroxide and antibiotic pastes on human stem cells, finding variations in the impact based on the type of medicament used.
Hoshino E, Kurihara-Ando N et al. [23]	Ciprofloxacin, Metronidazole, MinocyclinCalcium Hydroxide and Triple Antibiotic Paste (Concentration not specific (Triple Antibiotic Paste),	In vitro antibacterial susceptibility testing	Duration not specified	The study demonstrated the effectiveness of a combination of ciprofloxacin, metronidazole, and minocycline against bacteria from infected root dentine, highlighting its potential in endodontic treatment.
Kitikuson P et al. [24]	Triple Antibiotic Paste	Attachment ability of human periodontal ligament fibroblasts	Not specified	The study showed that triple antibiotic paste can affect the attachment ability of human periodontal ligament fibroblasts, which has implications for regenerative procedures.
Kontakiotis EG et al. [25]	Triple Antibiotic Paste	Various protocols in Regenerative Endodontic TherapyData analysis of clinical protocols	Not specified	The study analyzed various clinical protocols in regenerative endodontics, providing insights into the effectiveness of different treatment approaches.
Kim SG et al. [26]	Not specified (Comprehensive Review) Review of regenerative endodontics	-	Not specified	The study offered a comprehensive review of regenerative endodontics, summarizing key findings and best practices in the field.
Berman LH et al. [27]	(Comprehensive Review)	Analysis of Regenerative Endodontics Protocols	Not specified	The review provided a detailed discussion on regenerative endodontics, focusing on applying various medicaments and their outcomes.
Benz V et al. [28]	Antibiotic Pastes and Calcium Hydroxide	(Meta-analysis) Meta-analysis of regenerative endodontics outcomes	Not specified	The study presented a meta-analysis focusing on the outcomes of regenerative endodontics with antibiotic pastes and calcium hydroxide, identifying trends and effective treatment.
Murad M et al. [29]	Antibiotic pastes and Calcium Hydroxide	Promotion of dentin wall thickness and apical closure	Not specified	The study indicated that antibiotic pastes promote thicker dentin walls, while calcium hydroxide is more effective in fostering apical closure.
Vishwanat L et al. [30]	Not specified	Effect of bacterial biofilm on osteogenic differentiation of stem cells Not specified	Not specified	The study explored how bacterial biofilms influence the osteogenic differentiation of stem cells, providing insights into challenges in

				regenerative endodontics.
Hutton B et al. [31]	Not specified	(PRISMA-NMA Extension) Systematic review incorporating network meta-analysis.	Not specified	The study discussed the PRISMA-NMA extension for conducting systematic reviews with network meta-analysis, providing a framework for future research.
Deeks JJ, et al. [32]	Not specified	Analyzing data and undertaking meta-analyses	Not specified	The chapter provided guidelines for analyzing data and conducting meta-analyses in systematic reviews that are relevant to endodontic research.
Jain G et al. [33]	Various intracanal medicaments	Viability of stem cells of the apical papilla.	Not specified	The study evaluated the effectiveness of different intracanal medicaments on the viability of stem cells from the apical papilla, highlighting variations in outcomes.
Moranker R et al. [34]	Various intracanal medicaments	Viability, proliferation, and differentiation of stem cells	Not specified	The systematic review assessed the impact of various intracanal medicaments on stem cell viability, proliferation, and differentiation, providing an overview of their regenerative potential.
Jagdale S et al. [35]	Triple Antibiotic Paste	Coronal discoloration at varying depths and times	Not specified	The study compared coronal discoloration induced by two triple antibiotic revascularization protocols, providing insights into the aesthetic outcomes of these treatments.

CONCLUSION

Based on the systematic review of the effects of triple antibiotic paste (TAP) and calcium hydroxide (Ca(OH)₂) on the survival of apical stem cells, several important conclusions can be drawn. TAP, while effective in reducing microbial load within the root canal, can be detrimental to the survival of stem cells from the apical papilla (SCAP) when used in high concentrations. However, lower concentrations of TAP are less harmful and can even support the survival and proliferation of SCAP. On the other hand, calcium hydroxide (Ca(OH)₂) consistently positively impacts SCAP survival across various concentrations. It creates a favorable environment that promotes stem cell proliferation and differentiation, making it an ideal intracanal medicament for regenerative endodontic procedures. In summary, while both TAP and Ca(OH)₂ are effective intracanal medicaments, Ca(OH)₂ is more conducive to the survival and proliferation of apical stem cells, especially at higher concentrations. TAP, though beneficial, should be used with caution, paying close attention to its concentration to avoid cytotoxic effects on stem cells.

REFERENCES

- Ballal V, Kundabala M, Acharya S, Ballal M. Antimicrobial action of calcium hydroxide, chlorhexidine and their combination on endodontic pathogens. *Aust Dent J*. 2007;52(2):118-21.
- L.Krishna prasad ,Bhagat S Tanwar, Naveen Kumar.Comparison on Calcium Hydroxide & Triple

Antibiotic Paste(TAP)as intracanal medicament in emergency pain reduction.

- Vasiliki Taraslia , Dimitrios G , Prasanti ED, Margono A, Djauhaline N. Effect of Triple Antibiotic Paste(TAP), Calcium Hydroxide, Ledermix on the viability of pulp mesenchymal stem cells.Int Journal of Applied Pharmacology 2019 ,11;49-53.
- Hargreaves KM , Diogenes A, Teixeira FB. Treatment options : Biological basis of regenerative endodontic procedures.Journal of Pediatric Dentistry 2013 ,35: 129 -140.
- Shah N, Logani A ,Bhasker U. Efficacy of revascularization to induce apexification /apexogenesis in infected , non vital , immature teeth : A Pilot Clinical Study . Journal of Endodontics 2008 ,34 919 -925.
- Kumari, A., Sawhney, H., Kashwani, R., Gupta, G., & Das, S. J. Triple antibiotics: A synergistic approach to combating infection. <https://doi.org/10.18231/j.ijce.2023.036>
- Mohammadi Z, Dummer PM. Properties and applications of calcium hydroxide in endodontics and dental traumatology. *Int Endod J* 2011 Aug;44(8):697-730.
- Mandwe Ashish P, Patil Pravin G, Shetty Heeresh K, Shetty Vathsalya K, Pimpale Sandeep K. Role of triple

- antibiotic paste in revascularization and endodontics - a review. *Int J Contemp Med Res* 2015;2(2):186-190
- Anjaneyulu K, Nivedhitha MS. Influence of calcium hydroxide on the post-treatment pain in Endodontics: a systematic review. *J Conserv Dent* 2014 May;17(3):200-207.
 - Cehreli ZC, Isbitiren B, Sara S, Erbas G. Regenerative endodontic treatment (revascularization) of immature necrotic molars medicated with calcium hydroxide: a case series. *J Endod*. 2011 Sep;37(9):1327-30. doi: 10.1016/j.joen.2011.05.033. Epub 2011 Jul 13. PMID: 21846559.
 - Ruparel NB, Teixeira FB, Ferraz CC, Diogenes A. Direct effect of intracanal medicaments on survival of stem cells of the apical papilla. *J Endod*. 2012 Oct;38(10):1372-5. doi: 10.1016/j.joen.2012.06.018. Epub 2012 Aug 16. PMID: 22980180.
 - Pai S, Vivekananda Pai AR, Thomas MS, Bhat V. Effect of calcium hydroxide and triple antibiotic paste as intracanal medicaments on the incidence of inter-appointment flare-up in diabetic patients: An in vivo study. *J Conserv Dent*. 2014 May;17(3):208-11. doi: 10.4103/0972-0707.131776. PMID: 24944440; PMCID: PMC4056388.
 - Prasada, Lashkari. (2016). Comparison of Calcium Hydroxide and Triple Antibiotic Paste as Intracanal Medicament in Emergency Pain Reduction: In vivo Study. *IJO CR*.
 - Gougousis K, Giannakoulas DG, Taraslia V, Agrafioti A, Anastasiadou E, Kontakiotis EG. Number of Dental Stem Cells on Root Canal Dentin after Application of Triple Antibiotic Paste or Calcium Hydroxide: An In Vitro Study. *Eur J Dent*. 2019 May;13(2):161-165. doi: 10.1055/s-0039-1688733. Epub 2019 Sep 3. Erratum in: *Eur J Dent*. 2019 May;13(2):e1. doi: 10.1055/s-0040-1709090. PMID: 31480089; PMCID: PMC6777155.
 - Alasqah M, Khan SIR, Alfouzan K, Jamleh A. Regenerative Endodontic Management of an Immature Molar Using Calcium Hydroxide and Triple Antibiotic Paste: a Two-Year Follow-Up. *Case Rep Dent*. 2020 Feb 10;2020:9025847. doi: 10.1155/2020/9025847. PMID: 32095292; PMCID: PMC7035542.
 - Jamshidi D, Ansari M, Gheibi N. Cytotoxicity and Genotoxicity of Calcium Hydroxide and Two Antibiotic Pastes on Human Stem Cells of The Apical Papilla. *Eur Endod J*. 2021 Dec;6(3):303-308. doi: 10.14744/eej.2021.97658. PMID: 34967344; PMCID: PMC8842431.
 - Báez V, Corcos L, Morgillo F, Imperatrice L, Gualtieri AF. "Meta-analysis of regenerative endodontics outcomes with antibiotics pastes and calcium hydroxide. The apex of the iceberg". *J Oral Biol Craniofac Res*. 2022 Jan-Feb;12(1):90-98. doi: 10.1016/j.jobcr.2021.11.005. Epub 2021 Nov 10. PMID: 34815932; PMCID: PMC8593466.
 - Pai S, Vivekananda Pai AR, Thomas MS, Bhat V. Effect of calcium hydroxide and triple antibiotic paste as intracanal medicaments on the incidence of inter-appointment flare-up in diabetic patients: an in vivo study. *J Conserv Dent* 2014 May;17(3):208-211.
 - Ehrmann EH, Messer HH, Adams GG. of intracanal medicaments to postoperative pain in endodontics. *Int Endod J* 2003 Dec;36(12):868-875.
 - Metzler RS, Montgomery S. Effectiveness of ultrasonics and calcium hydroxide for the debridement of human mandibular molars. *J Endod*. 1989;15:373-378.
 - Cytotoxicity and Genotoxicity of Calcium Hydroxide and Two Antibiotic Pastes on Human Stem Cells of The Apical Papilla Davoud JAMSHIDI, 1 Mohamadreza ANSARI, 2 and Nematollah GHEIBI 3 *Eur Endod J*. 2021 Dec 31; 6(3): 303-308.
 - Hoshino E, Kurihara-Ando N, Sato I, Uematsu H, Sato M, Kota K, et al. In-vitro antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. *Int Endod J*. 1996;29(2):125-30
 - Kitikuson P, Srisuwan T. Attachment ability of human apical pious effect of triple antibiotic paste on human periodontal ligament fibroblasts. *Int Endod J*. 2014;47(8):769-75.
 - Kontakiotis EG, Filippatos CG, Tzanetakis GN, Agrafioti A. Regenerative endodontic therapy: a data analysis of clinical protocols. *J Endod*. 2015;41(2):146-54.
 - Kim SG, Malek M, Sigurdsson A, Lin LM, Kahler B. Regenerative endodontics: a comprehensive review. *Int Endod J*. 2018;51(12):1367-88
 - Berman LH, Hargreaves KH. Regenerative endodontics. In: Rostein I, editor. *Cohen's pathways of the pulp*. 12th ed. Canada: Elsevier; 2021. p. 476
 - Viviana Benz, Lorena Corcos . Meta-analysis of regenerative endodontics outcome with antibiotic paste & calcium hydroxide : The Apex of Iceberg. *Journal of Oral Biol Craniofac* 2022 Jan- Feb;12(1):90-98.
 - Mohammed Murad ,Monty S Duggal . Intracanal Antibiotic pastes may promote thicker dentin walls & calcium hydroxide may promote more apical closure. *Journal Evidence based Dental Practice* 2022 Jun 22(2) :101728
 - Vishwanat L., Duong R., Takimoto K., et al. Effect of bacterial biofilm on the osteogenic differentiation of stem cells of apical papilla. *J Endod*. 2017;43:916-922.
 - Hutton B., Catala-Lopez F., Moher D. The PRISMA statement extension for systematic reviews incorporating network meta-analysis: PRISMA-NMA. *Med Clin*. 2016;147(6):262-266.
 - Deeks Jonathan J., et al. Analysing data and undertaking meta-analyses. *Cochrane handbook for systematic reviews of interventions*. 2019:241-284.
 - Gaurav Jain, Akriti Goel et al. Evaluation of effectiveness of intracanal medicaments on viability of stem cells of apical papilla. *Journal Pharma Bioallied Sciences* 2020 Aug 12(suppl) S228-232.
 - Moranker Rahul ,Nitesh Tewari et al. In-vitro Evaluation Effect of Intracanal Medicaments on viability, proliferation & differentiation of stem cells from apical papilla :A Systematic Review. *Euro Endo J* 2022(7) 167-177.
 - Jagdale S, Bhargava K, Bhosale S, Kumar T, Chawla M, Jagtap P. Comparative evaluation of coronal discoloration induced by two triple antibiotic revascularization protocols when used at varying depths of temporary sealing material at the end of varying time periods. *J Conserv Dent*. 2018;21(4):388-93.
 - Lu YM, Qin JN. A comparison of the effect between Vitapex paste and antibiotic paste in apexification [Article in Chinese] *Shanghai Kou Qiang Yi Xue*. 2004;13(5):449-51.