Original Article

Evaluation of Effectiveness of Intracanal Medicaments on Viability of Stem Cells of Apical Papilla

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ABSTRACT

Introduction: Regeneration, in the field of endodontics, is the process of restoring and maintaining both architectural form and biological functions of damaged tooth. Presently, regenerative endodontics is not hypothetical and is an alternative to conventional apexification procedures. There is a deficient knowledge concerning the role of intracanal medicaments and their effect on dental stem cells. **Aim:** The aim of this study was to evaluate the effectiveness of commonly used intracanal medicaments on the viability of dental stem cells of the apical papilla (SCAPs). **Materials and Methods:** SCAPs were cultured and subjected to various concentrations including triple antibiotic paste, double antibiotic paste, Augmentin, and calcium hydroxide ($Ca(OH)_2$). Viable percentage of stem cell counts was obtained 3 days after treatment. **Results:** All three antibiotics used hereby significantly decreased SCAP cell survival at particular concentrations, whereas $Ca(OH)_2$ showed stimulating effect on SCAP survival. **Conclusion:** As per results obtained within limitations of this study, use of $Ca(OH)_2$ in regenerative endodontics in comparison to different commonly used antimicrobial combinations is recommended. Hereby, for clinical use, we suggest adequate concentrations of antimicrobials with adequate antibacterial efficacy should be used.

Keywords: *Calcium hydroxide, double antibiotic, regeneration, stem cells of apical papilla, triple antibiotic*

Introduction

Regeneration, in the field of endodontics, is the process of restoring and maintaining both architectural form and biological functions of the tooth that has been damaged, and in turn maintaining the vitality of the tooth. Repair on the other side not only restores the affected tissue but also replaces the damaged tissues, without return of their function.[1] Presently, the conventional endodontic treatment procedures do not regenerate the tooth but attempts to maintain the lifelike functions to resemble that of normal tissues.[2]

Teeth are natural source of stem cells, which have the ability of self-renewal and multidirectional differentiation. Dental stem cells can be obtained from periodontal ligament, dental pulp, and apical papilla. Previous documented literature shows numerous reports on significant advancements in understanding the biology of regeneration and knowledge of stem cells along with various factors such as growth factors, which are helpful in the differentiation of these pluripotent cells into various lineage of cell types.[3-9]

These days, regenerative endodontics is no longer a hypothetical approach but rather a practical alternative to conventional root apexification. This procedure depends on

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profuse chemical canal irrigation rather than mechanical root canal preparation and usage of combination of antibiotics.[10,11] Moreover, during revascularization, debridement and cleaning is not possible in root canal walls, thus both irrigation and intracanal dressings have a crucial role in pulpal cavity disinfection.

The biology behind this procedure involves the apical tissue cells activated due to the induced blood clot into the disinfected pulpal cavity. Thus, these apical stem cells in turn activate and helps in the formation of hard tissue inside the dentinal walls.[12] Therefore, root canal medicaments ideally should possess good antibacterial properties and should provide favorable environment for stem cells to regenerate.

Traditionally, commonly used material was calcium hydroxide ($Ca(OH)_2$) to attain a calcified barrier during apexification while treating an immature root. But due to certain drawbacks such as its high pH, it was initially not used for revascularization treatment, and at this time, triple antibiotic paste (TAP) was initially introduced.[13] Commonly used TAP during revascularization procedures is a combination of ciprofloxacin, metronidazole, and minocycline, and has been proven to be quiet efficient in infected root canal systems.[14-16] However, other combinations of intracanal medicaments such as that of metronidazole and ciprofloxacin (double antibiotic paste [DAP]) and the combination of metronidazole, ciprofloxacin, and cefaclor (modified triple antibiotic paste [mTAP]) have also been used in various studies.

Though numerous data are available, still lack of knowledge concerning the role of intracanal medicaments and their effect on dental stem cells persists. Thus, the aim of this *in vitro* study was to evaluate the effectiveness of commonly used intracanal medicaments on the viability of dental stem cells of the apical papilla (SCAPs).

Materials and Methods

This study was undertaken in the department of conservative dentistry and endodontics after prior approval from the institutional ethics committee. The viable apical stem cells were obtained from two extracted immature mandibular third molars, indicated for extraction after a prior informed consent from the patients. The extracted teeth were instantly washed and collected in sterile phosphate-buffered saline (PBS) and were stored until SCAP harvesting. Carious permanent third molars or with any pulp and periapical disease were excluded from our study.

Cell culture and preparation

SCAPs were isolated from the apical papillae of the immure permanent teeth by enzymatic digestion.[17] Cells were cultured using poly-D-lysine-treated 94-well culture plates for 24 h. The cell culture medium was removed, and it was again filled with 100 µL of test material per well. Cells obtained were then treated with 0.05% trypsin, and further again cultured onto subsequent culture plates. This was repeated till eight passages, and then SCAPs thus obtained were again inoculated.

The cells obtained were divided into two groups, namely control group and treatment group. Both the groups were treated using: (1) TAP (combination of metronidazole, ciprofloxacin, and minocycline in a 1:1:1 ratio), (2) DAP containing metronidazole and ciprofloxacin in a 1:1 ratio, (3) Augmentin, and (4) $Ca(OH)_2$. The SCAPs were exposed to inserts of drugs at the desired concentrations and were incubated at 37°C and 5% CO_2 for 3 days. On the third day, SCAPs obtained were washed with PBS followed by 0.05% trypsin for 3 min at 37°C. Later, we collected the cells by centrifugation method, and the cell supernatant was removed and suspended in a fresh medium. Viable cells in each treatment group were calculated by an automated cell counter after treatment with trypan blue.

Statistical analysis

The data thus obtained were recorded and tabulated and were sent for statistical analysis. One-way analysis of variance and Bonferroni *post hoc* test were used for comparisons. $P < 0.05$ was considered as statistically significant.

Results

TAP concentrations at 0.01, 0.1, 1, 10, and 100 mg/mL were evaluated for SCAP survival [Figure 1]. We observed that at 0.01 and 0.1 mg/mL concentrations, minimal or no effect was observed in the cell viability of SCAPs, whereas higher concentrations of 1, 10, and 100 mg/mL showed 58.0%, 8.0%, and 1.3% survival of SCAPs, respectively. This showed that as the concentration of TAP increased the viable population of the SCAPs decreased.

In relation to DAP, survival of 56%, 9%, and 1.2% SCAP was observed at 1, 10, and 100 mg/mL concentrations [Figure 2]. With Augmentin, survival of 55.5%, 8%, and 0.9% SCAP was observed at 1, 10, and 100 mg/mL concentrations, respectively [Figure 3]. On comparison, the difference in the results of the three antibiotic pastes used was found to be statistically not significant ($P > 0.05$).

With $Ca(OH)_2$, a significant increase in the survival of SCAPs accounting for 69% was observed at 1 mg/mL concentrations [Figure 4], whereas at rest, in case of other concentrations, minimal or no effect was observed. Overall, we did not observe any harmful

effect or decreasing percentage of survival of SCAPs as observed previously with the use of antibiotic pastes in our study.

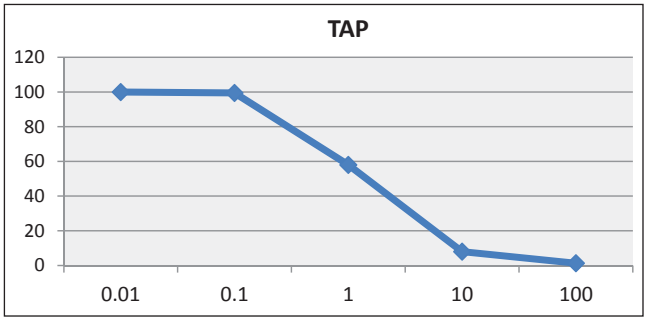

Figure 1: Graphical representation of survival of stem cells of the apical papilla after treatment with triple antibiotic paste at different concentrations

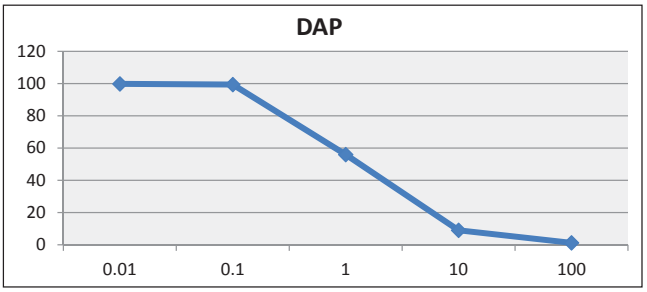

Figure 2: Graphical representation of survival of stem cells of the apical papilla after treatment with double antibiotic paste at various concentrations

Discussion

Successful revascularization of immature teeth with necrotic pulp is certainly challenging as it focuses on

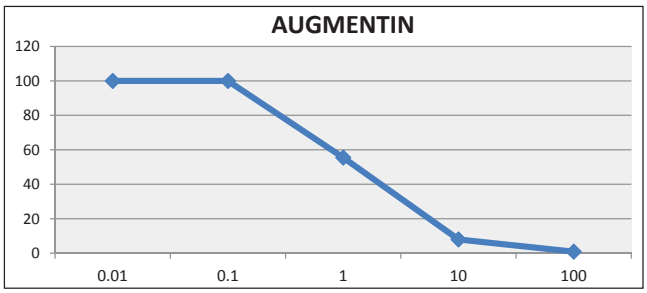

Figure 3: Graphical representation of survival of stem cells of the apical papilla after treatment with Augmentin at various concentrations

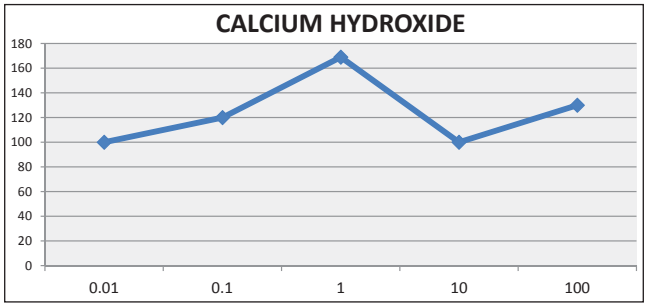

Figure 4: Graphical representation of survival of stem cells of the apical papilla after treatment with calcium hydroxide at various concentrations

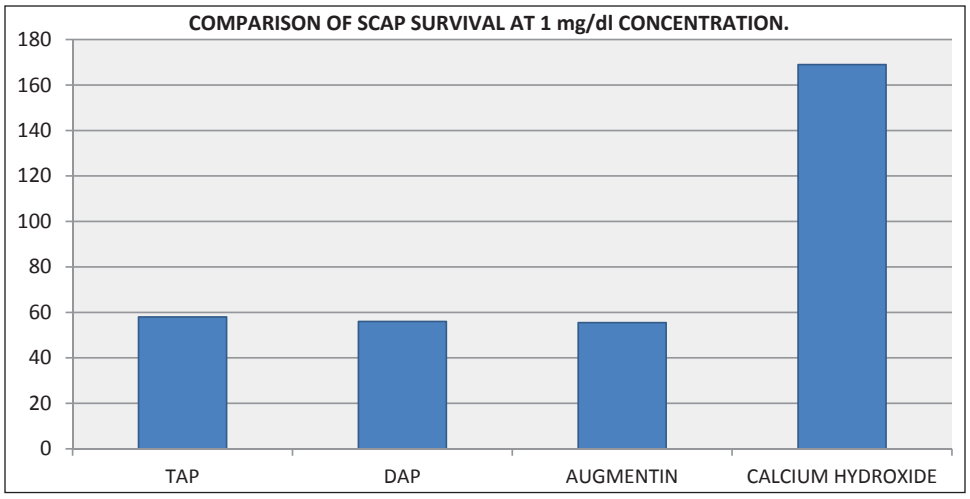

Figure 5: Comparison of the survival of stem cells of the apical papilla at 1 mg/dL concentration

Table 1: Comparison of survival of stem cells of the apical papilla after treatment with triple antibiotic paste, double antibiotic paste, and Augmentin at different concentrations

Medicament used	Percentage of survival of SCAPs at various concentrations used (mg/mL)				
	0.01	0.1	1	10	100
TAP	100%	99.5%	58%	8.0%	1.3%
DAP	99.8%	99.4%	56%	9%	1.2%
Augmentin	100%	100%	55.5%	8%	0.9%

disinfection within the pulpal cavity with a negligible detrimental effect on the SCAPs. Therefore, the viability or survival rate of these cells after treatment with antimicrobials within the canals has to be taken into consideration, if we are discussing in reference to the success of revascularization treatment. Hence, in our study, we have considered percentage viability of SCAPs as a referral for determining the effectiveness of the intracanal medicament on regeneration used in our study.

In this study, SCAP was used, which is commonly used in various regenerative studies, though on the contrary, some few studies by Pérez *et al.*[17] and Prasanti *et al.*[18] have also used dental pulp stem cells (DPSC). SCAPs have been reported to have a high proliferative capacity in comparison to DPSC; therefore, we preferred using it in our study.

Our results show that all the three medicaments used in our study, namely TAP, DAP, and Augmentin have harmful effects on the survival of stem cells [Table 1]. While interestingly, $Ca(OH)_2$, on the contrary, proves to have stimulating effects at higher concentrations on the stem cells. At a concentration of 1 mg/mL, a marked decrease in the survival of SCAP population is observed for TAP, DAP, and Augmentin, whereas $Ca(OH)_2$ shows a marked increase in its survival [Figure 5]. In consensus with our findings, similar results were also observed by Ruparel *et al.*[19]

Ruparel *et al.*[19] suggested that intracanal medicaments have influence on stem cells at two significant steps; first, at the time of diffusion into the apical papilla, which may affect stem cells within apical papilla, and second, the remaining antimicrobials may affect stem cells for the second time when carried within the papilla again during provoked bleeding.

On the contrary, Prasanti *et al.*[18] reported that both TAP and $Ca(OH)_2$ were observed to decrease the viability of DPSC. They reported that the cell survival with TAP at a concentration of 1 mg/mL was found to be greater than that of $Ca(OH)_2$ at the same concentration. Thus, TAP and $Ca(OH)_2$ were shown to have almost similar effects on the viability of DPSC.

Chuensombat *et al.*[20] reported that TAP was observed to be toxic on dental papilla cells at a given concentration of 25 μg/mL. This could be explained on the basis that the solubility of TAP is maintained for a longer time due to its low pH, which helps it to enter within the cell. This leads to its increased toxicity. Thus, it has been suggested that antibiotics having neutral pH and potent antibacterial properties should be preferred. Few other authors have also reported greater cytotoxicity of TAP as an intracanal medicament in regenerative endodontics.[19,21]

In literature review, TAP, on the contrary, has also been referred to as an effective disinfecting material and appropriate for vital tissue regenerative procedures. Bose *et al.*[22] reported that regenerative endodontic treatments with both TAP and $Ca(OH)_2$ significantly increased the root length in comparison to Mineral Trioxide Aggregate [MTA] apexification. Similarly, Lovelace *et al.*[23] also reported that TAP helps in inducing the growth of pluripotent stem cells into the canal space from periapical region.

Our results have shown $Ca(OH)_2$ to be beneficial in stem cell survival and even in promoting their growth. But on the contrary, authors such as Neha *et al.*[13] have discarded the use of $Ca(OH)_2$ for revascularization procedures because of its high pH, which might have damaging effect on the stem cells.

It has been observed that many studies use these medicaments in "thick paste" or a "slurry" form,[4,24-26] which provides a larger concentrations of around 1000 mg/mL USP grade of these antibiotics during the procedures. Our results do not favor the use of such concentrated solutions as they may have damaging effects on the stem cells.

Conclusion

Our results are in favor of using $Ca(OH)_2$ in regenerative endodontics in comparison to different commonly used antimicrobial combinations. Hereby, we suggest that for regenerative procedures, adequate concentrations of antimicrobials with adequate antibacterial efficacy should be used. But still further studies using regenerative models are required before the application of these medicaments in clinical practice.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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