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Effect of irrigants on proliferation & osteogenic potential of apical papilla stem cells

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Abstract

The study was done to assess &compare effect of irrigants on proliferation & osteogenic probablity of apical papilla stem cells. Current research was performed on16 cell samples (Stem cells from Apical Papilla) from 3rd molar from a single donor categorized into 2 groups with 8 samples in Group Aconsisting of MEM alpha medium with 20%Endocyn& Group B consisting of MEM alpha medium with 0.1%Octinidine dihydrochloride for evaluation of cell survival & alkaline phosphatase activity for 24 hours to 7 days' time period. Obtained data was evaluated statistically. The effectiveness of 20%Endocyn was significantly different from 0.1%Octinidine dihydrochloride on stem cells.

Keywords: Regenerative Endodontics, Octinidine dihydrochloride, osteogenic potential, Endocyn.

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1. Introduction

Due to trauma, caries, and other developmental abnormalities, developing dentition is susceptible to pulpal necrosis [1-3]. In cases of missing dentition, the loss of an immature permanent tooth can be catastrophic, leading to inadequate maxillofacial growth and a loss of aesthetic appeal. Historically, apexification techniques utilizing calcium hydroxide or MTA apical plugs were used to treat these teeth [4-5]. Apexification has no positive effect on root development but eliminates pathosis signs and symptoms. Therefore, a biologically based process known as "Regenerative Endodontics" was developed in 1960 based on the groundbreaking work of Dr. Nygard Ostby to restore damaged structures like dentine, roots, and pulp dentine complex cells. Regenerative endodontics is recommended for the treatment of immature permanent teeth with pulpal necrosis in younger patients (8–18 years old) because of the open apex's large diameter, which can promote tissue ingrowth, and its higher healing capacity [6]. Encouraging and permitting further root development is the aim. It involves the interaction of scaffolds, growth factors, and stem cells. Tissue function can regenerate when these three elements are purposefully altered [7]. The process involves first cleaning the canal area and then allowing stem cells to enter it [8]. A unique group of undifferentiated cells capable of both self-renewal and differentiation are known as stem cells. Mesenchymal cells known as stem cells of the apical papilla (SCAP) are usually found near the root end and have been shown to proliferate more quickly and differentiate more fully into odontoblast-like cells [9]. These stem cells may become nonviable, immobile in the target area, and less functional if they are subjected to common irrigants or medications [10-11]. An ideal irrigant for regenerative operations would be one that sterilizes the canal area while avoiding damage to nearby human cells [12]. Because of its capacity to disintegrate necrotic tissue and effectively break microbial biofilms, sodium hypochlorite (NaOCl) has shown to be a successful irrigant [13-14]. NaOCl's toxicity to SCAPs and periodontal ligament (PDL) cells, however, is a serious drawback [15]. Another effective endodontic irrigant that damages PDL but effectively gets rid of bacteria is chlorhexidine (CHX) [15-16]. SCAP attachment and survival are enhanced by 17% EDTA.

Conversely, 6% NaOCl + EDTA decreased cell viability and EDTA alone is not sufficient for effective disinfection. The search for ideal irrigant has devised the use of some new irrigants in Regenerative Endodontics like Endocyn&Octinidine Dihydrochloride (OCT).Endocyn, a pH-neutral mixture of hypochlorous acid and hypochlorite, is a superoxidized solution made of sodium chloride and clean water that has been demonstrated to possess antibacterial qualities.16 is intended to be used as an irrigant for teeth. Endocyn may out to be a helpful therapy for prostate regeneration [17].Octinidine Dihydrochloride (Octenisept)is basically an antiseptic introduced in 1990 for skin burns, wound disinfection.Itis a potential root canal irrigant with antibiofilm properties [18-19]. It is widely active against pathogens covering both Gram-positive and Gram-negative bacteria& fungi. 2% Phenoxyethanol, a preservative component in the irrigant has shown to be effective against oral bacterial like Streptococcus mutans and Actinomyces viscosus with no toxicity to tissues and acts in synergy with other antimicrobial agents [20].

2. Methodology

Cells were collected from mandibular 3rd molar from single donor with healthy gingiva & no PDL disease who went for extraction in various dental clinics. Cells were preserved in least necessary medium alpha (MEM alpha) containing 10% fetal bovine serum (FBS) at 37ºC and 5% CO_2. Cells were approved to adhere to 16 well plates with MEM alpha medium. Cell media was replaced in 8 well plates with fresh MEM alpha media consisting of 20%Endocyn labelled as Group A while0.1%Octinidinedihydrochloride with MEM alpha media in another 8 well plates labelled as Group B respectively.

2.1. Cell Proliferation

Cells were incubated for up to 9 days at 37ºC in 8 well plates with MEM aphamedia with 20% Endocynand MEM alpha mediawith 0.1%OctinidineDihydrochloridein other 8 well plates. Phosphate Buffer Solution was used three times to rinse adherent cells before fluorometric measurement was performed using CyQuant fluorescent dye (Molecular Probes, Eugene, OR). Using filters suitable for roughly 480 nm of excitation and 520 nm of emission, the fluorescence was measured using a Synergy 2 plate reader (Biotek Instruments, Inc.). Results show the means and standard deviations of eight samples during a 24-hour and seven-day timeframe.

2.2. Alkaline Phosphatase Activity

After being plated into 16-well plates, the cells were treated to 20% Endocyn and 0.1% Octinidine Dihydrochloride for a maximum of nine days each. After three PBS rinses, the cells were frozen in order to lyse them. Each well (200 mL per well) received 1 mg/mL of P-nitrophenol phosphate in 0.1 M diethanolamine (pH 8.3), and the wells were gently stirred for 30 minutes at 25 °C. After adding 500 mL of 0.75 N NaOH to halt the enzymatic colour process, the absorbance at 405 nm was measured using a Biotek Synergy. 2 plate reader (Biotek Instruments, Inc). Values represent the meansand SDs of 8 samples at 24hour period & 7 days.

2.3. Statistical Analysis

Eight samples were averaged for the quantitative analysis, and the means and SDs were compared to the control values for cells that had not been treated.Analysisof variance was used to compare the experimental groups, and $P < .05$ was deemed significant. The normality of the data was verified using either the 1- or 2-way analysis of variance test.

3. Results

Endocyn promoted better apical papilla cell proliferation at low concentration & is not toxic to apical papilla cells at conc of 20% for 24hr time period.Endocyn was appreciably less cytotoxic to SCAP cells after a prolonged exposure of 24 hours. However, present graph depicts that lower concentrationsupto 20% maintained for 7 days shows a decrease in cell viability.0.1% Octinidine dihydrochloride induced higher proliferation of apical papilla cells even after 24 hours & doesn't prove to be significantly toxic to stem cells after exposure for 7 days. A slight decrease in cell viability can be observed in graph in 3 days after exposure.However, there is considerable variation in cell viability among the two irrigants in 7 days as shown in Table 1 & Figure 1. Low doses of Endocyn don't inhibitSCAP cell alkaline phosphataseactivity. Inhibition become detectable atconcentrations of 20% from 3-5 days and after 7 days of exposure. Exposure with 10% Endocyn for 3 days considerably reduced alkaline phosphatase in contrast to 0.1% Octinidine Dihydrochloride as shown in the Figure 2 & Table 2. At 3 days, OCT induced higher alkaline phosphatase activity than Endocyn.

4. Discussion

Apexified teeth that have necrotic pulp and are immature are said to be in a halted developmental stage, with no further root growth and normal pulpal nociception.21 In several documented situations, regenerative endodontics has arisen as an alternative therapeutic approach with the goal of promoting normal physiological processes such as root formation, immunological competence, and normal nociception22. It is predicated on the ideas of tissue engineering, specifically the distribution of suitable cells, scaffolds, and growth factors in space [21]. This procedure requires the proper disinfection of canal to home stem cells that can be achieved through effective irrigation. Endodontic irrigant like Sodium Hypochlorite has proven to be advantageous due to its ability to dissolve necrotic tissue with proper disinfection of canal. However, it also results in damage to periodontal ligament cells with limited biofilm activity. An ideal irrigant in regenerative endodontics should include wide antimicrobial properties with no harm to the cells in vicinity[22].Hypochlorous acid is the active component of irrigants such as Endocyn. It has a strong bactericidal effect since it may break down protein membranes of bacteria [23]. According to the current investigation, at low doses (20%), endocyton proved to be less cytotoxic to SCAP.SCAP cells had enhanced cytotoxicity, particularly following a 24-hour treatment. It can be helpful for other endodontic operations as well as for disinfecting immature permanent teeth during regeneration therapies.

When there is a risk of irrigant extrusion, such as in perforations or open apex situations, it can become a safe choice for irrigants. However, low concentration for longer period of time (7day) proved detrimental to the stem cells proliferation. Low doses of Endocyndoesn't inhibit SCAP cell alkaline phosphatase activity at 24 hours period. However, this became detectable atconcentration of 20% after7 days of exposure in agreement to a previous study by Scott et al., (2017) [20-23].Newer antimicrobial agents like Octinidine dihydrochloride has been proposed in endodontics with wide range antimicrobial action but poor tissue dissolving properties. Octinidine dihydrochloride has been shown to be able to significantly reduce the colony-forming units at a depth of 400 µm in dentinal tubules [24]. The mode of action is bactericidal, adhering to the cell wall of micro-organisms causing cell death.This study states that cell viability is maintained at 0.1% OCT in accordance to the cell viability assays which revealed 0.1% OCT had the lowest cytotoxicity [25].These findings support those of the studies by Schmidt et al. (2016), which found that an OCT-based solution (Octenidol) promoted lower cytotoxicity of human gingival fibroblasts, and Coaguila-Llerena et al. (2019), which reported this effect on human periodontal ligament cells [26-27]. OCT's low cytotoxicity can be attributed to the absence of an amide and ester structure, which reduces metabolite formation. The cytotoxicity of the substance has a direct bearing on any detrimental effects on the cytoskeleton structure [28].Thealterations in the cell cytoskeleton were first noticeable in the OCT group at 2.02% dosages in the earlier work by Coguilla et al., [25]. But most of in vitro studies have used 0.1% OCT available in the market. So, Present study includes the use of 0.1% Octinidine dihydrochloride that can be used as root canal irrigant. In present study, OCT induced higher ALP activity in between 3 days period which is in agreement to previous study [25].Thus, in comparison to endocyn;OCT does not impair ALP activity&induced higher proliferation of apical papilla cells even after 24 hours.

Table 1: Cell survival in both groups.

Group	N	Mean	SD	P-value
Group A	8	0.031	0.051	0.013*
Group B	8	0.017	0.027	

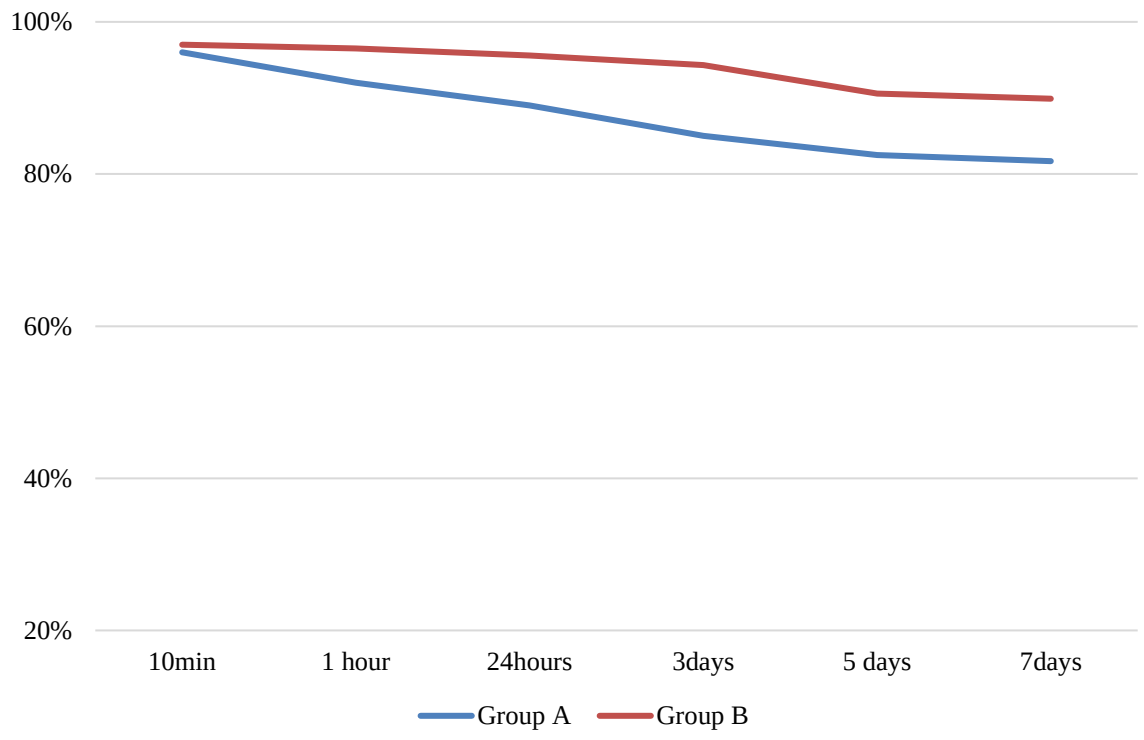

Figure 1: Cell survival in both groups.

Table 2: Alkaline phosphatase activity in both groups.

Group	N	Mean	SD	P-value
Group A	7	0.031	0.052	0.018*
Group B	7	0.029	0.040	

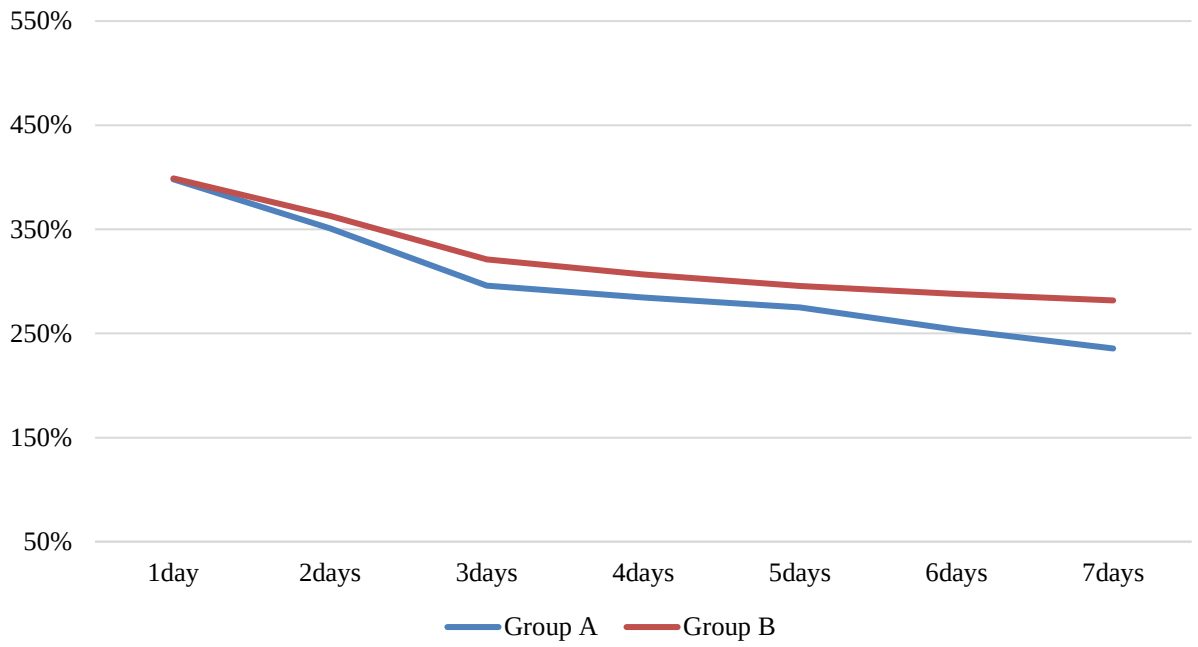

Figure 2: Alkaline phosphatase activity in both groups.

5. Conclusions

Compared to other conventional endodontic irrigants, Endocyn showed reduced cytotoxicity to SCAP cells in the periodontal ligament. Evaluating its advantageous irrigant qualities will require more research on its capacity to destroy biofilms, disintegrate tissue, and interact with existing endodontic irrigants. It has the potential to develop into a secure and efficient substitute for conventional endodontic irrigation and endodontic regeneration treatments. Compared to Endocyn, octenidine dihydrochloride has far less cytotoxicity. OCT did not inhibit ALP activity, and it only slightly altered the cytoskeleton, per earlier studies.Octinidine Dihydrochloride has to be carefully considered for use as a root canal irrigant in regeneration operations due to its lack of interaction with NaOCl, improved dentinal tubule disinfection, and capacity to maintain antibacterial efficacy even in the presence ofalbumin, blood, and mucus.

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