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Evaluation Of Tooth Discolouration Following The Use Of Silver Nanoparticle Based Intracanal Medicaments - An In Vitro Study

Research Article

KrishnaKanth Jaju[1], Iffat Nasim[2*]

[1] Department of Conservative Dentistry and Endodontics, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India.
[2] Professor and HOD, Department of Conservative and Endodontics, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India.

Abstract

Introduction: Optimal antibacterial efficacy of intracanal medicaments containing combinations of calcium hydroxide and silver nanoparticles has been well documented. However, concerns remain regarding the effect of silver based nanoparticles on tooth color. This study aimed to assess the effects of silver nanoparticles based intracanal medicaments on tooth color.
Material And Methods: Fifty extracted single-rooted,single-canal human teeth with straight roots, no caries, no cracks or fractures were collected and assessed. After cleaning and shaping of the root canals, the teeth were randomly divided into two experimental groups (n=20) Group A- Medicament prepared by combination of Calcium hydroxide and silver nanoparticles ,Group B- Medicament prepared by combination of Graphene oxide and silver nanoparticles and a control group of saline (n=10). After cleaning and shaping experimental groups were randomly divided into two equal groups and assigned medicament was applied below the cemento enamel junction (CEJ).The samples were incubated at 37°C and 100% humidity for one month. Color change (ΔE) was assessed using a spectrophotometer based on CIELAB system at four time intervals. T0-Before medicaments application , T1-Immediately after medicaments placement ,T2-After two weeks, T3-After one month. Data were analyzed using two-way and three-way ANOVA.
Results: There was no statistically significant differences in both the groups at baseline (T0) and after 15 days (T2) (p=0.775 and p=0.391, respectively).But there was a statistically significant difference between baseline (T0) and 1 month (T3) (p=0.037) in both the groups.
Conclusion: Both the tested intracanal medicaments caused tooth discolouration after a time period of one month. So its use must be limited to the root canal space only. Placement of intracanal medicament should be restricted for less than 15 days.

Keywords: Silver Nanoparticles; Cielab; Graphene; Spectrophotometry; Intracanal Medicament.

Introduction

Microorganisms are the main etiologic factors for the pulp and periapical diseases; therefore, the aim of endodontic treatment is complete elimination of microorganisms from the root canal system[1]. Studies have shown that cleaning and shaping of the root canal system in association with appropriate irrigation solutions do not decrease the microbial load due to presence of microorganism in inaccessible areas in the complex root canal system[2].

Enterococcus faecalis (E. faecalis) is the most resistant bacterial species that remains within the root canal system, It increases the failure rate of the root canal treatment[3]. The main reasons for using intracanal medicament is to prevent inter appointment bacterial proliferation and complete elimination of intracanal bacteria and accelerate recovery of periapical tissues[3,4]. The most commonly used intracanal medicament is calcium hydroxide (Ca (OH)2) and triple antibiotic paste, commonly used intracanal irrigant is chlorhexidine (CHX) and sodium hypochlorite[3-5]. The efficacy of each intracanal medicament, irrigants can be influenced by many factors such as serum proteins, collagen, pH and dentin etc. Majority of studies have shown that conventional root

***Corresponding Author**:
Iffat Nasim
Professor and HOD, Department of Conservative and Endodontics, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India.
E-mail: iffatnasim@saveetha.com

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canal disinfectants are unable to eradicate E. faecalis from the root canal space[3-6].

Recently, nanoparticles (diameter ≤100 nm) have gained popularity as antimicrobial agents[7]. Their greater surface area and charge density result in their greater interaction with the bacteria. Because of the novel physical and chemical properties of nanoparticles, recent studies have focused on using them for root canal disinfection[8]. The advantage of the use of nanotechnology is an increase in the surface-to-volume ratio of the materials, which increases the solubility, chemical activity and antibacterial efficacy of these agents as intracanal medicaments[8,9]. In endodontics, nanoparticles have been used as irrigants, intracanal medicaments or root canal sealers. Some of the materials which have exhibited improved properties with the application of nanotechnology are nanoparticles of zinc oxide, calcium hydroxide and silver[8-10].

The antibacterial properties of silver depend on silver concentration, release rate and its ability to bind to specific thiol groups containing sulfur and hydrogen in bacterial structures. The results of various studies have shown the superiority of silver based nanoparticles in terms of its biocompatibility[8-12].

Javidi et al. and Afkhami et al. highlighted the potential application of Ag-NPs mixed with calcium hydroxide as a root canal medicament[13]; however, despite the efficacy of Ag- NPs for root canal disinfection, their possible adverse effects such as tooth discoloration made them a controversial agent for in vivo usage especially for long term applications as a root canal medicament[13,14]. Previous studies have shown gray-black discolorations persistent to the crown due to the application of silver point as root canal filling material[13-15]. In addition, continuous release of silver ions resulted in recurrence of discoloration. Therefore, it may be speculated that Ag-NPs may have the same effect with respect to tooth discoloration[13-16].

Thus the question that arises from these experimental studies is whether the use of silver based nanoparticles as an intracanal medicament would result in tooth color change. Considering the gap of information on tooth discoloration due to the application of silver based nanoparticles, this study sought to assess the effect of novel silver based nanoparticles on tooth color.

Previously our team has a rich experience in working on various research projects across multiple disciplines [17-31] Now the growing trend in this area motivated us to pursue this project.

Material And Methods

Sample Preparation

The study protocol was approved by the institutional ethical committee.Fifty recently extracted single-canal human anterior teeth with straight roots, 22±1mm length, closed apices, no caries, cracks, fractures, restorations or resorption were included in this study. Presence of a single canal was ensured radiographically. Calculus and stains were removed by a scaler followed by the use of pumice paste and a polishing cup with a low-speed handpiece.

Standard straight-line access cavities were prepared using a round bur (DentsplyMaillefer, Ballaigues, Swit- zerland) and a fissure bur (DentsplyMaillefer, Ballaigues, Swit- zerland). A #15 K-file (Dentsply, Maillefer, Ballaigues, Switzerland) was introduced into the canal until its tip was visible at the apex and the working length was determined one millimeter short of this length. Each canal was prepared using ProTaper rotary system (DentsplyMaillefer, Ballaigues, Switzerland) and Dentsply X- smart plus motor (DentsplyMaillefer, Ballaigues, Switzerland) according to the manufacturer's instructions (S1, S2, F1- F3).

RC-Prep (Premier Dental Products, Norristown, PA, USA) was used as lubricant and 10 mL of 2.5% sodium hypochlorite (NaOCl) was used as irrigant during root canal preparation. A final rinse with 3mL of 17% ethylenediaminetetraacetic acid (EDTA) and 5.25% NaOCl was carried out for five minutes for smear layer removal. Each canal was then rinsed with 5 mL saline and dried with paper points. To prevent leakage of materials through the apex, the apices of all the teeth were sealed with self-cure glass ionomer (GC Dental, Tokyo, Japan).

Apical two-thirds of the roots were mounted in acrylic resin. The samples were randomly assigned to three groups:

Group A (n=20): Medicament prepared by combination of Calcium hydroxide and silver nanoparticles.

Group B - Medicament prepared by combination of Graphene oxide and silver nanoparticles.

Control group (n=10): Root canals were filled with saline.

The medicaments were cautiously applied into the Root canal (below the CEJ); a cotton pellet was placed into the pulp chamber and the access cavity was temporarily restored with Cavit (3M ESPE, St. Paul, MN, USA). During the experiment, all the teeth were kept in a dark glass container with a piece of cotton covering them. The container was incubated at 37°C and 100% humidity for one month.

Assessment Of Tooth Color

Tooth color in all groups was analyzed at four time points-Baseline (T0), immediately after medicament placement(T1), Two weeks after medicament placement (T2), one month after medicament placement(T3).

Color assessment was done in a laboratory at 37°C temperature. A piece of white non-fluorescent leneta paper was used as the background as recommended by the manufacturer. Samples were fixed on a jig. Collimated light source (tungsten) illuminated the tooth surface at 45°angle relative to the vertical axis, and the spectrophotometer (CS-2000, Konica Minolta, Japan) was adjusted at 0° angle relative to the vertical axis at 70 cm distance from the tooth surface with 0.1° viewing angle. This adjustment created a circular measurement area with an approximate diameter of 1.2 mm at the center of samples. The spectrophotometer was calibrated prior to measurement of each sample and color of each sample was measured in triplicate at the center of the marked square and the mean of measured values was calculated and reported.

Color coordinates were calculated using D65/2º viewing condition in CS-S10W software. This viewing condition is commonly used in aesthetic dentistry assays. The CIELAB color space was

used for the analysis. Other colorimetric data including chroma (C*), hue angle and tristimulus values were also reported by the aforementioned software. Color differences were calculated using CIELAB 1976. After obtaining the , L* a*and b* parameters, ΔL* Δa*,and Δb* were calculated. Color differences (ΔE*) was calculated using the following equation -

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

Data were analyzed using two-way and three-way ANOVA. Post hoc ANOVA was applied whenever required. The level of statistical significance was set at 5%.

Results And Discussion

Mean of ΔE*, ΔL*, Δa* and Δb*values for all groups are represented in tables 1-3, respectively.".

There were no statistically significant differences in both the groups at baseline (T0) and after 15 days (T2) (p=0.775 and p=0.391, respectively).But there was a statistically significant difference between baseline (T0) and 1 month (T3) (p=0.037).

Tooth color can be assessed using several techniques and visual inspection is among the most commonly used techniques. Several standard methods are available for visual inspection of tooth color[32]. In the simplest technique, the specimen and a standard shade guide or the Munsell color chips are compared by the same observer under the same standard light source[32,33]. However, color perception is variable among different individuals and even in the same person at different time points; thus, this technique is associated with errors[32-34]. In addition, the color spectrum of these shade guides is limited. Use of colorimetric devices seems to be more accurate[35]. Devices used for this purpose are divided into two main groups of colorimeters, which determine the three color coordinates and are not very reliable and spectrophotometers, which measure the transmitted or reflected light and determine the color parameters via the use of mathematical formulations[36]. Color assessment by these devices eliminates the subjective errors of color analysis. Spectrophotometry is commonly used for measurement of transmitted or reflected light and is suitable for color analysis of convex and asymmetric objects like teeth[36,37]. Spectrophotometers are the reference tools for color assessment and are superior to other techniques for dental applications[36-38]]. In the current study, CS2000 Konica Minolta spectrophotometer was used for color assessment of tooth crowns. Also, the CIELAB color space was applied for assessment of discoloration of teeth. The L*, a*and b*parameters stand for brightness, red-green axis and yellow- blue axis, respectively[36-39]. This system measures color as a numerical value and shows the overall color change or ΔE as a scalar value. Since the mean colorimetric coordinates at baseline are different among groups, statistical analysis of these values results in incorrect interpretation of changes[36-40]. Thus, ΔE*,ΔL*, Δa*,Δb* and are calculated since they are more reliable for assessment of changes at each time point; therefore, this system was used in this study. The smear layer is composed of microcrystalline and organic debris and covers the root canal walls after root canal instrumentation. Presence of smear layer affects dentin permeability and subsequently the tooth color change[41]. Presence of smear layer decreases tooth discoloration. Under these conditions, longer time is required for the discoloration to occur[41,42]. The smear layer was eliminated in the current study in order to eliminate its effect on the results and to allow better penetration of medicaments into dentinal tubules. In endodontics medicaments are used in between treatment session for necrotic teeth, revascularization

Table 1: Mean of ΔL*, Δb* and Δa* values for all groups.

Groups	L value				A value				B value			
	Pre op	Immediate Post op	15 days	1 month	Pre op	Immediate Post op	15 days	1 month	Pre op	Immediate Post op	15 days	1 month
Ca(OH)2+Ag	86.9	87.5	88.9	90.8	1.7	2.6	3.6	4.1	23.2	24.4	24.9	25.3
Go+Ag	88.2	89.5	90.7	91.6	2.9	3.9	4.4	5.6	25.4	25.6	25.8	26

Table 2: Mean of ΔE* values for all groups

ΔE Value			
Groups	Immediate Post Op	15 days	1 month
A- Ca(OH)2+Ag	1.61	3.24	5.03
B- Go+Ag	1.65	2.92	4.38

Table 3: There were no statistically significant differences in both the groups at baseline (T0) and after 15 days (T2) (p=0.775 and p=0.391, respectively).But there was a statistically significant difference between baseline (T0) and 1 month (T3) (p=0.037).

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
IMMEDIATE POST OP	Between Groups	.004	1	.004	.093	.775
	Within Groups	.183	4	.046		
	Total	.187	5			
AFTER 2 WEEKS	Between Groups	.141	1	.141	.924	.391
	Within Groups	.611	4	.153		
	Total	.752	5			
AFTER 1 MONTH	Between Groups	1.092	1	1.092	9.471	.037
	Within Groups	.461	4	.115		
	Total	1.554	5			

and apexification treatments[43]. Thus, in the current study, we assessed the effects of medicaments on tooth color after variable time intervals to assess tooth discoloration following the short term and long term applications of medicaments.

Calcium hydroxide is used in different forms in endodontic procedures. For greater efficacy, it may be used in conjunction with some other materials such as Ag-NPs, which have shown promising results for efficient elimination of microorganisms from the root canal system[44,45]. Graphene is a carbon based flat monolayer arranged in a two dimensional hexagonal structure. It has distinguished mechanical, electrochemical and physical properties[46]. The graphene family nanomaterials has several graphene derivatives, such as Few-Layered Graphene (FLG), ultrathin graphite, Graphene Oxide (GO), reduced Graphene Oxide (rGO) and graphene nanosheets. It has a remarkable antibacterial ability against both Gram-negative and Gram-positive bacteria as studied by various researchers[47]. Graphene acts as a nano-knife, penetrates and cuts cell membranes of bacteria, induces mechanical stress, extracts phospholipids from lipid membranes and produces oxidative stress through ROS generation and by charge transfer phenomena[48]. However, some concerns exist regarding their potential to cause tooth discoloration. This issue must be cleared prior to clinical application of this agent.

In a clinical trial conducted by Day et al., application of Ultracal XS CH medicament in replanted teeth caused significant darkening of teeth in the clinical setting[49]; however, this color change only caused concerns in a few people. Their results were in line with our findings. Akcay et al. reported no significant color change in teeth due to the application of calcium hydroxide medicament (no significant difference with controls). Such variability in results may be attributed to different medicaments used since they applied calcium hydroxide mixed with distilled water, which has a different composition than graphene based silver nanoparticles used in our study[49,50]. Moreover, their experiment was conducted on bovine teeth; whereas, we used freshly extracted human teeth to better simulate the clinical setting. Similarly, Kim et al. showed tooth discoloration is due to the application of medicament to the access cavity and thus, recommended that application of medicaments must be limited to the root canal space[49-51].

In the current study tooth discolouration was seen in both the tested intracanal medicament, long-term application of both the tested intracanal medicaments caused significant change in tooth color. However,the medicament containing combination of graphene oxide and silver nanoparticles caused less tooth discolouration as compared to the medicament containing combination of calcium hydroxide and silver nanoparticles.

Our institution is passionate about high quality evidence based research and has excelled in various fields[52-62].

Conclusions

Within the limitations of present study, the following conclusions were drawn: Materials used as intracanal medicaments have the potential to change the tooth color. So, use of intracanal medicaments should be restricted for less than 15 days. Esthetic considerations must be taken into account as well as functional properties and therapeutic efficacy while selecting an intracanal medicament particularly for the anterior teeth. Combination of graphene oxide and silver nanoparticles caused less tooth discolouration as compared to the medicament containing combination of calcium hydroxide and silver nanoparticles.However, application of the intracanal medicament must be confined to the root canal and the residues in the pulp chamber must be carefully removed before restoring the crown.

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