Comprehensive Assessment Of Intracoronal Stains Induced Via Triple Antibiotic Paste, Mineral Trioxide Aggregate And Calcium Hydroxide Powder Combined With Aquatic Solution Of Chlorhexidine- An In Vitro Study

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Abstract

Tooth discoloration caused by various materials and agents causes internal stains, rendering the visual outcome of the pulp-treated tooth unappealing. Thus, a continuous impingement of agents in dentinal tubules causing tooth discoloration is expected to be the primary focus of this study. Furthermore, some speculated that tooth discoloration may not be caused by tubule penetration but rather by endodontic material remnants in the pulp chamber that darken over time and transmit their shade through hard tissues. Sixty premolars were chosen and randomly assigned to one of four groups, one of which was a control group. Four different agents were used to obtain internal stains. These samples were then sealed in distilled water at 37°C for three months using restorative Glass Ionomer Cement. All measurements were taken with a Spectrophotometer at the start and after 3 months. After 3 months of incubation, all intracanal agents induced intracoronary stains. After three months of incubation, the following values were collected: group I, group II, group III, and group IV. When compared to the other groups, the MTA group (group II) induced the most stains.

Keywords: Tooth discoloration; Calcium hydroxide; Chlorhexidine; Mineral Trioxide Aggregate; Antibiotic paste; Spectrophotometer

INTRODUCTION:

Aesthetics play a significant role in patient satisfaction following treatment. A tooth's color is influenced by both its intrinsic color and the presence of any extrinsic stains that may form on its surface. The absorption of scattered light by a tooth's enamel and dentine determines its intrinsic color. Dentine's absorptive properties are important in determining the overall color of a tooth. Endodontic treatment can result in tooth discoloration due to pulpal hemorrhage, residual pulp tissue, and therapeutic intracanal agents. Internal tooth staining may also be caused by oburating materials and temporary filling materials, in addition to intracanal agents. Endodontic materials ingressing into dentinal tubules during root canal therapy is thought to be the primary cause of progressive tooth discoloration.[1] Visible crown discoloration may not be caused by tubule penetration, but by endodontic material remnants in the pulp chamber that darken over time and transmit their colour through hard tissues. The extent of tooth discoloration is determined by the removal of the endodontic smear layer. It has been reported that the presence of this smear layer reduces dentine permeability.[2] With the growing prevalence of pulp-periapical pathologies, Triple Antibiotic Paste, Mineral Trioxide Aggregate, and Calcium Hydroxide in combination with Chlorhexidine are being used as therapeutic intracanal agents for resolving endodontic lesions as a non-surgical approach.

TAP (triple antibiotic paste) contains metronidazole, ciprofloxacin, and minocycline. One such antibiotic combination has shown promising results in endodontic therapy. Despite the fact that this antibiotic combination produces promising results, it causes discoloration of the coronal tooth structure. [1]

Mineral Trioxide Aggregate has a substantial amount of research data on its physical and biological properties. Given the growing demand for cosmetics, biomaterials should be chromatically stable, have optical properties similar to dental structures, and not stain hard dental tissues. According to recent studies, both White and Gray MTA formulations can cause tooth discoloration. From this standpoint, every new MTA-based material must be tested in terms of aesthetic and colour objectives. [1]

Because of its ability to induce hard tissue formation, broad antibacterial spectrum, and ability to act as a physical barrier to prevent root canal super infection, calcium hydroxide Ca (OH)2 plays an important role in endodontics. [3]

Chlorhexidine is effective against Ca (OH) 2-resistant bacteria, particularly E. faecalis. This property supports the use of CHX in endodontic retreatments, where such bacteria are commonly found. However, clinical studies have shown that CHX can stain teeth, especially when used as a mouth rinse for periodontal treatment. The most widely accepted staining mechanism for CHX-induced tooth discoloration appears to be non-enzymatic browning and the formation of pigmented black metal sulfides.[3]

MATERIALS AND METHODS:

Aim and objective of this study was to induce intracoronal stains via calcium hydroxide powder combined with aquatic solution of Chlorhexidine, mineral trioxide aggregate and triple antibiotic paste.

Source of samples: Sixty sound premolars extracted for orthodontic purpose were selected for the study. This study was conducted in the Department of Pedodontics and Preventive Dentistry, Karnavati Dental College and hospital, Uvarsad, Gujarat. Procedure: A total of sixty premolars extracted for orthodontic reasons were stored in distilled water at 37°C until the study was performed.

Criteria for Inclusion:

- Intact premolars extracted for orthodontics reasons.
- Teeth having sound structure.
- Teeth that had closed apices.
- Teeth free of any internal or external stains.
- Teeth without any trauma.

Criteria for Exclusion:

- Teeth with internal or external root resorption.
- Teeth having open apices.
- Traumatized teeth.
- Teeth having any kind of internal or external stains

These samples were divided equally (n= 15) into four groups including three experimental groups and a control group as follows:
Group I- Calcium hydroxide mixed with Chlorhexidine
Group II- Mineral Trioxide Aggregate
Group III- Triple Antibiotic Paste
Group IV- Control Group

All samples had access opened and pulp extracted before biomechanical preparation using the Crown Down Technique. To induce staining, different intracoronal agents were placed in the coronal half of the root in each study group. These samples were then sealed in distilled water at 37°C for three months using restorative Glass Ionomer Cement. All samples were removed from the distilled water after three months. Glass Ionomer Restorations were removed with diamond burs, and samples were visually inspected for intracoronal staining by a single-blind examiner. The samples were spectrophotometrically analysed, and the baseline T0 values of each group were compared. The readings were all recorded for statistical purposes. The mean and standard deviation calculated from the specimens for each group were compared using one-way ANOVA, the Bonferroni post hoc test, and Eta square. The level of significance in the current study was set at p0.05.

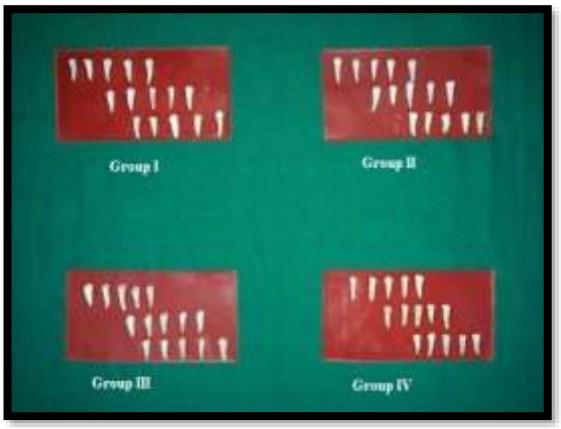

Fig 1: Showing all four group division

Fig 2: Showing placement of calcium hydroxide mixed with chlorhexidine solution

Fig 3: Showing placement of TAP

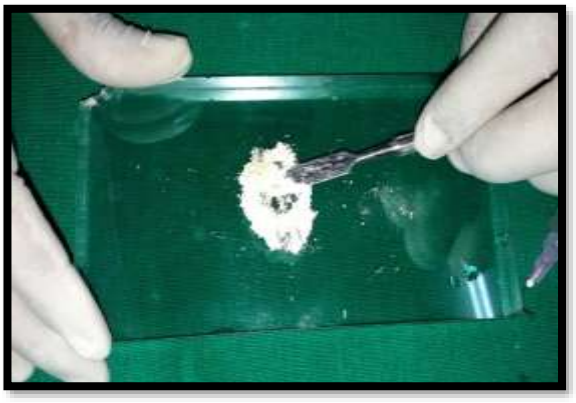

Fig 4: Showing mixing of MTA

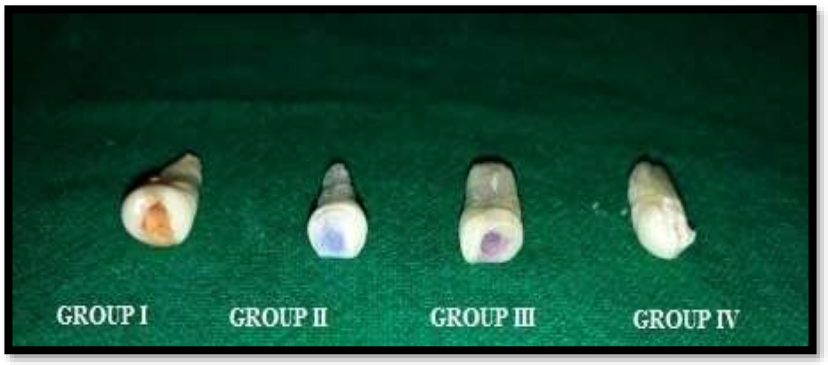

Fig 5: Shows intracoronal stains after 3 month

RESULTS AND DISCUSSIONS:

The present in vitro study was carried out in order to evaluate different intracoronal stains induced via Triple Antibiotic Paste, Mineral Trioxide Aggregate and Calcium hydroxide.

Table 1: The descriptive analysis of ANOVA, the Bonferroni post hoc test, and the Eta square of the T0 group are shown. Initially, all of the groups were separated and stored in distilled water. It was assumed that all teeth had the same colour index, but in this study, the baseline data showed a difference in colour index after storage in distilled water. As a result, the null hypothesis was rejected. Similarly, Paini T RD et al 27 concluded in a study on stimulating pulpal necrosis inducing discoloration in storage media that remnants of pulp in tooth after extraction lead to necrosis and discoloration of tooth. Furthermore, the Bonferroni post hoc test reveals a colouring index from major to minor as Group II>I>III>IV. In addition, Eta square test showed significant difference in color index among baseline groups.

Table 1

	Group	N	Mean	Std. deviation	Std. Error	95% Confidence Interval for Mean		ANOVA			Bonferroni post hoc test	Eta square
						Upper bound	Lower bound	F	df	P value		
T0	I	15	30.70	1.90	0.49	29.64	31.75	886.07	3	0.00	II>I>III>IV.	0.97
	II	15	44.93	.88	0.22	44.44	45.42					
	III	15	25.73	.98	0.25	25.19	26.28					
	IV	15	23.02	1.02	0.26	22.45	23.59					

Table 2: The descriptive analysis of ANOVA, the Bonferroni post hoc test, and the Eta square of the T1 group are shown. After applying intracanal agents and storing them in distilled water for three months, different groups showed different colour indexes, with Group II (MTA) showing the most discoloration (87.42). Furthermore, the Bonferroni post hoc test revealed a colouring index from maximum to minimum as Group II>I>III>IV, indicating that the lowest discoloration was present in the empty group.

Table 2

	Group	N	Mean	Std. deviation	Std. Error	95% Confidence Interval for Mean		ANOVA			Bonferroni post hoc test	Eta square
						Upper bound	Lower bound	F	df	P value		
T1	I	15	64.79	0.77	0.20	64.36	65.22	7137.31	3	0.00	II>I>III>IV.	0.99
	II	15	87.42	0.98	0.25	86.87	87.96					
	III	15	53.02	0.96	0.25	52.48	53.55					
	IV	15	41.53	0.85	0.21	41.06	42.00					

DISCUSSION:

One of the most common reasons for a patient to seek dental care is tooth discoloration. Discolored teeth are both cosmetically and psychologically unappealing. Understanding the etiology of tooth discoloration can help dental practitioners explain the exact nature of the condition to their patients. The mechanism of staining may have an effect on the outcome of treatment offered in some cases, and it also influences treatment options offered by dentists to their patients.

Extrinsic and intrinsic causes of tooth discoloration are distinguished by the location of stains. Extrinsic discoloration occurs on the surface of the tooth or within the acquired pellicle, whereas intrinsic discoloration occurs when chromogens are deposited into the bulk of a tooth, which can be of local or systemic origin.

Periapical pathologies are frequently encountered during endodontic therapy and are treated with a variety of intracanal agents. Calcium Hydroxide in combination with Chlorhexidine and Triple Antibiotic Paste, Mineral Trioxide Aggregate, and other therapeutic intracanal agents are used to treat endodontic lesions using a non-surgical approach.

At the end of three months, intracoronal discoloration of tooth was observed after placement of Calcium Hydroxide combined with an aquatic solution of chlorhexidine. Some studies have concluded that calcium hydroxide does have a discoloration potential, which may not have been adequately considered up to this point.[3-9] Although the exact mechanism of calcium hydroxide staining is unknown, it has been suggested that it is more likely due to any additional components, such as bismuth carbonate, which is commonly found in many commercially prepared calcium hydroxide pastes. Pure calcium hydroxide powder has yet to be explained convincingly.

Since a long time, calcium hydroxide powder combined with chlorhexidine has been used as an intracanal agent to treat periapical pathology, but its efficacy needs to be checked after a certain amount of time given due to its ability to cause crown discoloration as one of its possible side effects, as described by.[5,6,8] Also a study by researcher, described the potential for discoloration by calcium hydroxide mixed with chlorhexidine.[10] Furthermore, long-term use of calcium hydroxide for primary tooth treatment causes discoloration.[9]

MTA was used as one of the intracanal agents in this study, and it caused the most discoloration at the end of three months. The presence of bismuth oxide in MTA components is the primary cause of discoloration. A similar study was conducted by an author with result showing that how bismuth oxide present in Mineral Trioxide Aggregate causes tooth discoloration.[11] A study with different intracanal agents was conducted, and it was discovered that MTA caused equal discoloration in their experimental groups after three, six, and twelve months with or without the presence of blood contamination.[2] Additionally, a few researchers concluded from their experiment that White MTA induced grey discoloration of crowns of teeth in the presence of blood contamination in root canals. Similar studies have discovered that MTA can cause coronal tooth discoloration after prolonged use.[13-17] On contrary to that MTA is also used during apexification to form an apical block in the tooth.[18] A researcher described coronal tooth discoloration caused by TAP, gray MTA and white MTA but no discoloration was observed in biodentine. Hence biodentine is preferred as esthetic material.[19]

TAP (triple antibiotic paste) containing ciprofloxacin, metronidazole, and minocycline. Several studies have found that this mixture has good antimicrobial properties in infected root canals. Further research revealed that it is biocompatible.[20] Despite these advantages, several case studies have revealed that minocycline causes visible crown discoloration. Amoxicillin, cefaclor, and doxycycline have recently been proposed as antibiotic alternatives to minocycline for use in combination with ciprofloxacin and metronidazole. TAP is made by combining equal parts powdered antibiotics with saline or sterile water. This paste is left in the canal for three weeks. One unintended consequence of TAP use is discoloration of the tooth structure, particularly the crown, resulting in an unsightly appearance.

A study found that using bonding agents prior to TAP could help reduce coronal tooth discoloration.[21] Similarly, studies were carried out to compare tooth discoloration caused by TAP with that caused by various other materials.[22-26] They came to the conclusion that TAP caused discoloration after prolonged use. The current study discovered that the triple antibiotic paste causes coronal tooth discoloration after six months.

CONCLUSION:

The current in vitro study was designed to identify various intracoronal stains induced by various intracanal agents. After 3 months, the MTA group had the most discoloration, followed by the Calcium hydroxide mixed with Chlorhexidine and Triple Antibiotic Paste groups, and the control group had the least discoloration. Furthermore, the baseline data in this study showed different readings, which could have been another area of research as the storage media to induce staining or discoloration. As a result, more research is needed to gain a better understanding of the media as well as the agents.

REFERENCES

1. Ioannidis K., Mistakidis I., Beltes P., Karagiannis V. Spectrophotometric analysis of crown discolorationinduced by MTA- and Zn OE-based sealers. Journal of Applied Oral Science2013;21(2):138-144.
2. Lenherr P., Allgayer N., Weiger R., Filippi A., Attin T., Krastl G.Tooth discoloration induced by endodonticmaterials: a laboratory study. International Endodontic Journal 2012; 45:942–949.
3. Kontogiannis T. G., Koursoumirs A., Kerezoudis N. P.In vitro evaluation of the staining effects of two intracanal agentss over a 3-month period. Open Journal of Stomatology 2014; 4: 381-388.
4. Sulieman M., Addy M., MacDonald E., Rees J. S.The effect of hydrogen peroxide concentration on the outcome of tooth whitening: an in vitro study. Journal of Dentistry 2004; 32: 295–299.
5. Zanatta F. B., Antoniazzi R. P., Rösing C. K. Staining and calculus formation after 0.12%chlorhexidine rinses in plaque-free and plaque covered surfaces: a randomized trial. Journal of applied oral science 2010;18(5):515-21.
6. Day P. F. et al. Discoloration of teeth after avulsion and replantation: results from a multicenter randomized controlled trial. Journal of Endodontics2011;37(8):1052–1057.
7. Thomson A. D., Athanassiadis B., Kahler B., Walsh L.Tooth discoloration: Staining effects of various sealers andagentss. Australian Endodontic Journal2012; 38: 2–9.
8. Vatanpour M., Esmaili S., Dar P. M., Pour H. Z. Effects of green tea, calcium hydroxide and chlorhexidineon chemical structure and tooth discoloration. Bioscience Biotechnology Research Communications2017;10(2):202-207.
9. Xavier S. R., Pilownic K. J., Gastmann A. H., Echeverria M. S., Romano A. R., Pappen F. G. Bovine tooth discoloration induced by endodontic fillingmaterials for primary teeth. International Journal of Dentistry2017:1-5.
10. Lazzari J. et al. Spectrophotometric analysis of internal bleaching of traumatized teeth with coronal discoloration following regenerative endodontic procedures. Brazilian Journal of Oral Science 2022;21:1-11.

11. Marciano M. A., Duarte M. A. H., Camilleri J. Dental discoloration caused by bismuth oxide in MTA in the presence of sodium hypochlorite. Clinical Oral Investigations2015;19(9):1-10.
12. Felman D., Parashos P. Coronal tooth discoloration and white mineral trioxide aggregate. Journal of Endodontics 2013;39: 484–487.
13. Camilleri J. Staining Potential of Neo MTA Plus, MTA Plus and Biodentine used for pulpotomy procedures. Journal of Endodontics2015;14(7):1–7.
14. Akbari M., Rouhani A., Samiee S., Jafarzadeh H. Effect of dentin bonding agent on the prevention of tooth discoloration produced by mineral trioxide aggregate. International Journal of Dentistry2012:1-3.
15. Belobrov I., Parashos P. Treatment of tooth discoloration after the useof white mineral trioxide aggregate.Journal of Endodontics2011;37(7):1017–1020.
16. L.E. Pelepenko et al. Dental discoloration caused by Grey-MTAFlow cement: analysis of its physicochemical, biological and antimicrobial properties. Journal of Applied Oral Science 2020:1-15.
17. Wan-Chun Yang.et al. Tooth discoloration and the effects of internal bleaching on the novel endodontic filling material SavDen MTA. Journal of Formosan medical association (2021) 120: 476-482.
18. Kabaktchieva R., Gateva N. Vital pulpotomy in primary teeth with mineral trioxide aggregate (MTA).Journal of International Medical Association Bulgaria2009;2:102-108.
19. Kohli M. R., Yamaguchi M., Setzer F. C., Karabucak B. Spectrophotometric analysis of coronal tooth discoloration induced by various bioceramic cements and other endodontic materials. Journal of Endodontics 2015;41(11):1–5.
20. Hoshino E. et al. In-vitro antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. International Endodontic Journal 1996;29(2):125-130.
21. Shokouhinejad N., Khoshkhounejad M., Alikhasi M., Bagheri P., Camilleri J. Prevention of coronal discoloration induced by regenerative endodontic treatment in an ex vivo model. Clinical Oral Investigation Journal2017:1-7.
22. Iriboz E., Öztürk B. A., Körklü S., Tarcin B., Berker Y. G., Öveçoğlu H. S. Comparison of intracoronal bleaching methods on teeth discolored by different antibiotic pastes. Nigerian Journal of Clinical Practice2017;20(6):701-706.
23. Santos L. G. P., Felippe W. T., Souza B. D. M., Konrath A. C., Cordeiro M. M. R., Felippe M. C. S. Crown discoloration promoted by materials used in regenerative endodontic procedures and effect of dental bleaching: spectrophotometric analysis. Journal of Applied Oral Science2017;25(2):234-242.
24. Porter M. L. A., Munchow E. A., Albuquerque M. T. P., Spolink K. J., Hara A. T., Bottino M. C. Effects of novel 3d antibiotic-containing electrospun scaffolds on dentin discoloration. Journal of Endodontics 2016;42(1):106–112.
25. Akcay M., Arslan H., Yasa B., Karvik F., Yasa E. Spectrophotometric analysis of crown discoloration induced by various antibiotic pastes used in revascularization. Journal of Endodontics2013;40(6):1–4.
26. Rana B. K., Sohali K., Kiyani A. Antibacterial Efficacy and Discoloration Potential of Topical Antibiotics Used in Regenerative Endodontic Therapy. Found Uni J Dent 9;2(1):71-77.
27. Paini T RD et al. Technique for Darkening of Extracted Teeth Simulating Pulpal Necrosis Discoloration. Clinical, cosmetic and investigational dentistry. 2022:(14) 103–112.999