



ORIGINAL ARTICLE

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Comparison of the degree of coronal pigmentation in extracted pre-molars after the application of bi-antibiotic and tri-antibiotic pastes

Abstract: Objective: To compare the degree of pigmentation in coronal extracted premolars after applying bi-antibiotic and tri-antibiotic pastes. Method: Intentional non-probability sampling. A total of 30 healthy premolars which were extracted for orthodontic reasons and met the inclusion criteria were divided into 2 experimental groups and a control group of 10 teeth each. Group I (G1) was treated with bi-antibiotic paste, group II (G2) with tri-antibiotic paste; and group III or control (G3) was treated with saline solution. Two observers previously calibrated took the initial color sample from each tooth by giving them a value corresponding to the shade guide. After access opening and instrumentation, all teeth received the appropriate medication and color records were taken under the same initial light conditions for 30 days after the procedure. The obtained data were compiled into Excel for further descriptive and inferential analysis with SPSS 19.0. ANOVA test was used with a confidence level of 95%. Results: The degree of pigmentation was 1 ± 0.9 in G1, 12.2 ± 1.2 in G2 and 0.9 ± 0.8 in G3. No statistically significant differences were found between G1 and G3 ($p=0.8086$), but there was between G1 and G2 ($p<0.001$), and G2 and G3 ($p<0.001$). Conclusion: The degree of pigmentation with tri-antibiotic paste was higher than the degree of pigmentation with bi-antibiotic paste used as temporary medication. No difference was found between the degree of pigmentation in the group treated with bi-antibiotic and the control group treated with saline solution. Bi-antibiotic paste did not show pigmentation in the crown of the extracted premolars.

Keywords: *Coronal pigmentation, double antibiotic paste, triple antibiotic paste.*

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INTRODUCTION.

For dentists, treating teeth with immature apex and pulp necrosis is a challenge due to tooth fragility after a conventional apexification procedure. These teeth have a high degree of cervical radicular fracture for years after treatment¹.

Revascularization is a new conservative alternative for regeneration of necrotic pulps in teeth with immature apex. Revascularization of the pulp space in a necrotic tooth infected with apical periodontitis was believed to be impossible². Revascularization is an optional treatment

for permanent teeth with open apex which examines the maximum capacity of the pulp for dentin apposition and production of a stronger mature root to give more support against forces causing fractures^{3, 4}. However, the current protocol has potential clinical and biological complications, including pigmentation of crown, development of resistant bacteria and allergic reactions to medication^{5,7}.

Bacteria of the tissues are one of the main causes of periapical pathology because they remain in the root dentin or periradicular tissues. Using antibacterial drugs can be a method for eradicating bacteria in the treatment of

radicular canal^{8,9}. Metronidazole has an excellent activity against anaerobic bacteria in odontogenic abscess, but none against aerobic microorganisms¹⁰. In vitro investigations conducted in 1993 by Sato *et al.*¹¹ concluded that combining antibiotics is very effective for the sterilization of carious lesions, necrotic pulps and radicular dentin of deciduous teeth. In 1996, this conclusion was bolstered by Hoshino *et al.*¹² who demonstrated that the use of a single antibiotic did not have the same in vitro antibacterial effectiveness against bacteria of the infected dentin, pulps, and periapical lesions. However, when combined, these medicines were able to sterilize all the samples.

Tri-antibiotic paste composed of ciprofloxacin, metronidazole, and tetracycline (minocycline) has proven to be an excellent option for disinfecting radicular canal⁶. When used as a protocol to manage open apices, it has given successful results achieving revascularization³, which was corroborated by Banchs and Trope¹³. But tetracycline causes pigmentation in the crowns of treated teeth^{7,14}.

Regarding coronal pigmentation, using Ledermix[®] paste (Lederle Pharmaceuticals GmbH, Wolfratshausen, Germany) has proved that this intracanal medication contains tetracycline which discolors teeth⁵. Although this coronal discoloration is not often revealed in literature, it is known to be related to tetracycline. In 2008, these findings led Reynolds *et al.*⁵ to present a technique to avoid this permanent pigmentation in cases of revascularization. It consists in putting fluid resin at coronal level to protect dentinal tubules and prevent the pigmentation produced by tri-antibiotic paste while maintaining the revascularization potential of the pulp.

There is a new protocol in the revascularization procedure using bi-antibiotic paste which contains ciprofloxacin and metronidazole without tetracycline to prevent teeth discoloration¹⁵. While it has achieved successful revascularization in some cases, there are not related studies showing that bi-antibiotic paste does not pigment teeth.

The objective of this study was to compare in vitro the degree of pigmentation in coronal extracted premolars after applying bi-antibiotic and tri-antibiotic pastes.

MATERIALS AND METHOD.

After approval by the Ethics Committee of the Research Subsystem of the Universidad Metropolitana (ORD: No. 054) and obtaining the signature of the informed consent on the part of the patients who would donate the teeth for this study, it was proceeded to carry out the experiment.

Initially, this in vitro study consisted of 50 upper and lower premolars extracted from patients between 13 and 16 years of age in the Oral Surgery Clinic at the Faculty of Dentistry of the Universidad Metropolitana in Barranquilla. Teeth were cleaned from all the rest of organic tissue with 5.25% sodium hypochlorite and maintained in physiological saline solution until the experiment began. Sampling was non-probabilistic and intentional. A total of 30 teeth were chosen taking into account the following inclusion criteria: healthy upper and lower premolars, free of decay or restorations, without anatomical defects and extracted due to orthodontic reasons. Exclusion criteria were the following: pigmented teeth, with prior endodontic treatment, with restorations or with coronal fracture. Teeth were randomly divided into two experimental groups and a control group of 10 teeth each and soaked in 2% sodium hypochlorite for one hour in order to remove organic debris. Prophylaxis was performed on the crowns with a pumice stone and rubber cup to remove exogenous pigments and obtain the natural tooth color.

Color was obtained using Ivoclar[®] Shade Guide (Chromascop Universal Shade Guide-Vivadent, USA) (Figure 1). It comprises 5 categories of shades: 1 (01, 1A, 2A, 1C), 2 (2B, 1D, 2C, 3A), 3 (3A, 5B, 2E, 3E), 4 (4A, 6B, 4B, 6C) and 5 (6D, 4C, 3C, 4D). The shade guide was ordered from lightest to darkest and was assigned a number in the appropriate position to allow statistical analysis. Each increase of darkness was considered as a degree and the values can be seen in Table 1.

For recording color of the dental crowns, the color was selected for each tooth before starting the pilot phase in the same room and under the same ambient lightning conditions. A wooden white box reflecting neutral-white light of 6,500 K with a wavelength between 330-770nm from a lamp

Table 1. Shade order.

Scale	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Code	01	1A	2A	1C	2B	1D	1E	2C	3A	5B	2E	3E	4A	6B	4B	6C	6D	4C	3C	4D

The shade guide was ordered from lightest to darkest and was assigned a number in the appropriate position to allow statistical analysis. Each increase of darkness was considered as a degree.

Figure 1. For registering color, tones from Ivoclar® Chromascop Universal Shade Guide- Vivadent USA. were used. It comprises 5 shade categories: 1(01, 1A, 2A, 1C), 2 (2B, 1D, 2C, 3A), 3 (3A, 5B, 2E, 3E), 4(4A, 6B, 4B, 6C) and 5 (or 6D, 4C, 3C, 4D).



with a 40W bulb (Philips®, USA) was used as light source. The crown was isolated with hygienic dental dam® (Coltene/Whaledent, Switzerland) for an optimal shoot. The evaluation was carried out in a double-blind at the beginning and 30 days later by two examiners previously calibrated who worked independently. They reevaluated their decision when there was no agreement.

Access opening was performed in ovoid form in the occlusal surface of the premolars, starting with a #4 diamond bur (SS White®, USA) and removing all the roof of the chamber with a #4 carbide bur (SS White®, USA). The length of the canal was determined surpassing the apex with a #10 file and subtracting 1mm from this length. Then, they were instrumented with a manual #25 Flexofile file (Dentsply® Maillefer, Ballaigues, Switzerland). They were irrigated with 5.25% sodium hypochlorite, 2ml after each instrument using a 10ml hypodermic syringe and 23 gauge needles. The canal entrance was widened up to the middle third with Gates Glidden burs #2 to #6 (Dentsply® Maillefer, Ballaigues, Switzerland) to keep the same canal entrance size for all the samples. Teeth were dried with an aspiration catheter (Ultradent®, USA) and randomly assigned into three groups of 10 teeth each.

Three groups were organized for the application of pastes:

G1: ten samples of premolars were treated with bi-antibiotic paste which was applied to the pulp chamber. The paste was composed of 500 mg ciprofloxacin MK® (Technochemical plants, Colombia) and 500 mg metronidazole MK® (Technochemical plants, Colombia) and physiological saline as a vehicle. Initially, bi-antibiotic paste was prepared by removing the layer of sugar of the pills with a scalpel blade and separately grinding antibiotics in mortars. Each antibiotic became fine dust by combining equal quantities of antibiotics in a mixture with physiological saline in a Dappen dish with a clean spatula until obtaining a homogeneous pasty mixture of creamy consistency. Bi-antibiotic paste was introduced to the duct with a lentulo from the cemento-enamel junction level until the apex. All paste excesses were cleaned with saline-moistened cotton pellet. When the entrance into the chamber was completely clean, a dry cotton ball was placed and the opening was sealed with a plug of temporary Coltosol® (Coltene/Whaledent, Switzerland). Bi-antibiotic paste was kept within the root canal for 30 days.

G2: It consisted of 10 samples of premolars for which the same opening, instrumentation and irrigation process was followed. 100mg of minocycline® (Lafrancol, Colombia) was added to this mixture to make tri-antibiotic paste which was placed with lentulos inside the canals which were temporarily sealed as in the previous group.

G3: It comprised 10 samples of premolars. As the two previous groups, it underwent the same procedure for opening and preparation of the canal. A saline-moistened cotton ball was placed and they were sealed with temporary Coltosol® (Coltene/Whaledent, Switzerland). Because it was the control group, intracanal medication was not used.

Teeth were kept in an INE 500 precision micro processed laboratory incubator (Memmert®, USA) at 37°C, 100%

Figure 2. Comparison of samples treated with bi-antibiotic paste at the beginning and 30 days later.



Figure 4. G3. Control of the samples treated with physiological saline solution at the beginning and 30 days later.



Figure 3. G2. Control of the samples treated with tri-antibiotic paste at the beginning and 30 days later.



relative humidity over a 30-days period the experiment lasted. When the time was fulfilled, the temporary filling was removed with a #4 carbide bur (SS White®, USA) and the cotton ball was eliminated with a DG-16 endodontic explorer. Then, the paste was withdrawn by irrigating with 5.25% NaOCl and using #25K files ((Dentsply® Mai). Afterwards, the canal were dried with paper points from the Second Series (New Estetic®, Colombia) and a wet cotton ball was placed to allow its hardening. Finally, they were sealed with a temporary filling of Coltosol® (Coltene/Whaledent, Switzerland).

Color recording were made under the same lighting conditions as the initial decision to make the comparison. The value of the color of the teeth was registered and the difference between the initial color and the color after 30 days was observed using the shade guide mentioned above as reference (Figures 2, 3 and 4). The analysis focused on color differences seen in each tooth. The obtained data were collected in an Excel table and were statistically analyzed

Table 2. Pigmentation values for each group.

G1			G2			G3		
C1 VALUE	C2 VALUE	C2 - C1	C1 VALUE	VALUE C2	C2 - C1	C1 VALUE	C2 VALUE	C2 - C1
2	3	1	4	14	10	1	2	1
1	2	1	1	15	14	1	1	0
2	3	1	2	14	12	1	2	1
3	3	0	3	14	11	2	2	0
1	3	2	1	13	12	1	2	1
3	3	0	1	13	12	1	3	2
2	5	3	1	15	14	1	3	2
3	3	0	1	13	12	1	3	2
2	3	1	4	17	13	1	1	0
2	3	1	1	13	12	1	1	0
Average		1	Average		12.2	Average		0.9
Standard deviation		0.943	Standard deviation		1.229	Standard deviation		0.876

with SPSS 19.0 program. ANOVA test was used for both the descriptive and inferential analysis with a confidence level of 95%.

RESULTS.

Mean values of the degree of pigmentation in the groups were 1 ± 0.9 in G1, 12.2 ± 1.2 in G2 and 0.9 ± 0.8 in G3 (Table 2).

No statistically significant differences were found between G1 and G3 ($p=0.8086$), but between G1 and G2 ($p<0.001$), and G2 and G3 ($p<0.001$).

DISCUSSION.

Tooth pigmentation is a negative effect in revascularization when using tri-antibiotic paste because it contains tetracycline. The same findings were discovered by Reynolds *et al.*⁵ in 2008 when they reported a technique to avoid teeth changing color by using a coating on the walls of the pulp chamber at coronal level with fluid resin before placing the medication. They used tri-antibiotic paste containing tetracycline with positive results because the resin avoided direct contact at the coronal pulp level. However, the application of fluid resin is not easy and cannot control the extension of the material beyond what is desirable insi-

de the duct.

Hargreaves *et al.*¹⁶ reported a series of revascularization cases, including those of a 13-year-old girl who presented pulp necrosis and chronic apical abscess. They conducted 5 weekly appointments without instrumentation and irrigated only with 5% sodium hypochlorite and 3% hydrogen peroxide, leaving the tooth open to allow drainage between the first and second appointment. They used metronidazole and ciprofloxacin (bi-antibiotic paste) in the second appointment as intracanal medication. After 6 weeks, vital tissue was observed in the channel. They applied calcium hydroxide in the chamber and then protected it with cement glass ionomer and resin composite. After 15 months, they conducted pulpar tests with positive electrical response. Apical closure with thickening of the walls was observed 30 months later.

In 2009, Trope¹⁷ suggested changing tetracycline for Arestin® in tri-antibiotic paste. He showed photos with less coloration of the tooth, but with changes in the tone. Being Arestin another type of tetracycline, he recommends using bi-antibiotic paste containing only ciprofloxacin and metronidazole. In revascularization processes, it has been seen that periradicular bone can be repaired by irrigating with sodium hypochlorite, then placing calcium hydroxide paste

and sealing the chamber with cement glass ionomer. This procedure can be used instead of applying tri-antibiotic paste to increase wall thickness in the duct and achieve apical closure^{18,19}.

Although using calcium hydroxide in revascularization processes does not cause pigmentation of the crown, authors such as Banchs and Trope¹³ do not recommend it. They have suggested that calcium hydroxide can kill any type of remaining pulpar cells, including stem or progenitor cells that are presented in the tissue of the dental pulp or possibly alter the apex and its resident stem cells, which is critical for the continued development of the root. Also, Bose *et al.*²⁰ mentioned that calcium hydroxide is effective for extending radicular length, only if it is placed short of working length of the root, and suggested that the material is not near the apical tissue, where the progenitor cells are located and can interfere with the revascularization process.

In fact, bacteria in the root canals must be eliminated during treatment to achieve a successful outcome that would allow healing of the periapical diseases in adults. For this purpose, an antibacterial drug alone may not be effective, even if it has a broad antibacterial spectrum, because the bacterial composition of the infected canals is complex. Therefore, selection of antibacterial drugs should be based on the most up-to-date information on bacteriological knowledge. It is necessary to use a paste with a combination of antibiotics that have great bactericidal power and do not cause adverse reactions in patients¹⁸.

García-Godoy and Murray²¹ mentioned that calcium hydroxide and other disinfectants should be used for revascularization only when it can be proved that sodium hypochlorite was enough to disinfect the necrotic pulp tissue.

Comparación de la pigmentación coronal en premolares extraídos después de aplicar pastas Bi-antibiótica y Tri-antibiótica.

Resumen: Comparar el grado de pigmentación coronal en premolares extraídos después de aplicar pastas Bi-antibiótica y Tri-antibiótica. Método: Muestreo no probabilístico e intencional. Se seleccionaron 30 premolares sanos extraí-

dos por motivos ortodónticos, que cumplieran los criterios de inclusión, distribuidos en 2 grupos experimentales y un grupo control de 10 dientes cada uno. El grupo I (G1) fue tratado con pasta Bi-antibiótica, el grupo II (G2) con pasta Tri-antibiótica; y el grupo III o control (G3) fue tratado con suero fisiológico. Dos observadores previamente calibrados tomaron el color inicial a cada muestra dándoles un valor

They concluded that an antibiotic paste can be used as a disinfectant together with sodium hypochlorite and patients should be alerted about the potential of pigmentation. Bearing in mind that throughout history of endodontic treatment it has been shown that cleaning the canals is more important than the medicines, but these also help, one must choose those which have less negative effects.

Bi-antibiotic paste has clinically shown to possess disinfection properties in the root canal and success in cases of revascularization¹⁵⁻¹⁷. However, there are not comparative studies related to pigmentation because of using bi-antibiotic paste in revascularization processes in literature.

Recently, several clinical case reports demonstrated that, in spite of periapical abscess formation and periradicular bone resorption as a result of root canal infection in immature teeth, conservative revascularization treatment can allow root development and maturation¹⁷.

The results of this study show a significant difference in the degree of pigmentation using tri-antibiotic paste when compared to bi-antibiotic paste. Considering that both revascularization techniques are effective and that aesthetic has a very important role in dentistry, we recommend conducting similar studies with a larger number of samples and with more time to confirm its use.

CONCLUSION.

The degree of pigmentation with tri-antibiotic paste was higher than the degree of pigmentation with bi-antibiotic paste used as temporary medication. No difference was observed between the degree of pigmentation with bi-antibiotic paste and the control group treated with saline solution.

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correspondiente a la guía de colores. Luego de la apertura e instrumentación, todos los dientes recibieron los medicamentos correspondientes por 30 días, tomándose registros de color con las mismas condiciones iniciales de luz. Los datos obtenidos se recopilaron en Excel para analizarlos de forma descriptiva e inferencial con el programa SPSS 19.0, se utilizó el test de ANOVA con un nivel de confianza del 95%. Resultados: El grado de pigmentación fue de $1 \pm 0,9$ en G1, $12,2 \pm 1,2$ en G2 y $0,9 \pm 0,8$ en G3. No se hallaron diferencias estadísticamente significativas entre G1 y G3 ($p=0,8086$), si

las hubo entre G1 y G2 ($p<0,001$), y G2 y G3 ($p<0,001$). Conclusión: El grado de pigmentación con la pasta Tri-antibiótica fue superior al grado de pigmentación de la pasta Bi-antibiótica utilizada como medicación temporal. No se observó diferencia entre el grado de pigmentación presente de la pasta Bi-antibiótica y el grupo control tratado con suero fisiológico. La pasta Bi-antibiótica no presentó pigmentación de la corona de los premolares extraídos.

Palabras clave: *Pigmentación coronal, pasta doble anti-biótica, pasta triple antibiótica.*

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