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REVIEW

Mechanisms of Staining with Demeclocycline and Doxycycline Root Canal Medicaments

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ABSTRACT

Tetracyclines are a unique class of antibiotics which also have additional effects including anti-inflammatory, anti-resorptive and substantive within the root canal. There has been a long-held view that tetracycline medicaments discolour teeth and should be avoided. The evidence base around this topic was explored, including a review of the methodology used in laboratory studies. A search of PubMed, Medline and Scopus databases was conducted to identify studies of demeclocycline and doxycycline medicaments used in root canal therapy. An analysis of the methodology used in these studies was performed to determine if these replicate current clinical practice. The related literature on mechanisms of tetracycline stability and the effects of light, oxidation, moisture and chemical interactions was examined. Studies investigating the effects of Ledermix paste on segments of bovine dentine and avulsed or reimplanted teeth as well as combinations with other antibiotics were excluded from this review. Even though demeclocycline medicament pastes were introduced in 1962, the first laboratory studies of discolouration were not done until 2000. All later studies followed a similar approach, which included exposure to sodium hypochlorite for up to 30 minutes and storage in moist conditions with 100% humidity. Staining during dark storage and enhanced staining on exposure to light were reported, indicating multiple pathways of degradation of demeclocycline and its reaction products. Light, moisture and oxidation are the key factors which drive discolouration from demeclocycline. Clinical issues from tooth staining can be prevented by removal of medicament pastes from the access cavity, and placement of a sound interim restoration. Use of a doxycycline paste obviates concerns of staining. Laboratory assessments of the potential for staining should replicate in vivo conditions.

Keywords: Endodontic medicaments, oxidation, staining, tetracyclines

HIGHLIGHTS

- TThe non-antimicrobial property of tetracyclines is a significant advantage that is not seen in other antibioticsused in root canal medicaments, such as clindamycin.
- All six previous in vitro studies on the staining effectsof Ledermix paste have used the same methodology,which does not replicate clinical conditions.
- The chemistry of tetracycline discolouration and thecase reports highlight that breakdown in the accesscavity in interim and final restorations allows moistureand oxygen to enter the coronal and radicular parts ofthe tooth and initiate discolouration when Ledermixpaste is used.
- The removal of all existing restorations, cracks, and caries prior to commencing root canal treatment and asound interim restoration that will remain in place forthe period of the root canal treatment, followed by adefinitive coronal restoration after the root filing, willprevent discolouration with Ledermix paste.
- Because the systemic use of doxycycline does not discolour teeth, its use in medicaments that also containanti-oxidants will obviate problems of discolouration, even if the access cavity breaks down.

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INTRODUCTION

Intracanal medicaments that contain a tetracycline antibiotic can, in some circumstances, stain the coronal and radicular parts of teeth; this has been a long-held view in endodontics and has been discussed by clinicians and manufacturers of dental materials. Nevertheless, investigators have not observed this clinically (in either short- or long-term applications), situations where excess medicament has been removed from the coronal aspects of the access cavity, and there has been a sound coronal restoration in the access cavity, preventing the entry of fluid. This raises the discrepancy between perception and reality. The purpose of this paper is to discuss the aetiology of discolouration from tetracycline medicaments and to determine under what particular situations staining can occur. Demeclocycline and doxycycline are tetracycline drugs

commonly used in dentistry. Demeclocycline is a major therapeutic component of Ledermix paste (Lederle Pharmaceuticals, Wolfsratshausen, Germany), a medicament paste that was first introduced in 1962. Ledermix paste contains 3.2% demeclocycline hydrochloride and 1% triamcinolone acetonide in a polyethylene glycol base. It is used widely in parts of Europe as well as in Australia, New Zealand, Oceania, and elsewhere (1, 2). With regard to other tetracycline antibiotics, doxycycline hyclate at a concentration of 3% is present in MTAD irrigant solution (Dentsply, Tulsa, USA) (3) and in Doxymix (Ozdent, Sydney, Australia). The latter is a medicament paste that contains the same vehicle as Ledermix paste, with doxycycline as the active antibiotic ingredient rather than demeclocycline (4).

Tetracycline endodontic medicaments have therapeutically important non-antibacterial properties that provide additional benefits for their use in endodontics. The anti-resorptive properties of Ledermix paste have been attributed to its corticosteroid that works synergistically with the tetracycline (5) to reduce external inflammatory resorption following the replantation of avulsed teeth. Other additional non-antibacterial properties of tetracyclines include their ability to inhibit matrix metalloproteinases, mop up free radicals, inhibit the excessive inflammatory response to antigenic stimuli, reduce reactive oxygen species, stimulate new bone formation, and provide substantive antibacterial actions. Such benefits are not seen with other antibiotics used in endodontic medicaments, such as clindamycin. In tissue, clindamycin has a shorter half-life (4 h) than either demeclocycline (10–12 h) or doxycycline (18–24 h) (6-8), and clindamycin lacks non-antibacterial properties.

The long-held view that tetracycline medicaments cause staining is based on the outcomes of the systemic administration of tetracycline antibiotics to young patients during periods of tooth formation, when they become incorporated into the tooth structure and from previous in vitro studies. Tetracyclines can be divided into two groups based on their propensity to cause discolouration of teeth from systemic use, namely, those that cause severe tooth discolouration (e.g., demeclocycline and tetracycline hydrochloride) and those that cause little discolouration (e.g., oxytetracycline and methacycline). With any agent, the effects depend on the dose as well as the duration and time of administration (9-11). The systemic use of doxycycline in children <8 years of age does not cause staining or enamel hypoplasia of the developing teeth (12, 13).

There have been seven laboratory studies of staining from Ledermix paste placed into the root canal system, with six studies using human teeth (4, 14-18) and one using bovine teeth (19). Ledermix paste has been used since 1962; however, it was only in the year 2000 that the first studies of this type were conducted (15, 16). Later investigations have followed a similar protocol, with only minor changes to the methodology. It is now timely to review what is known regarding the chemistry of discolouration from Ledermix paste and other products in order to establish the clinical implications. In parallel, a number of issues with past laboratory research into this topic will also be identified in this paper.

A search of PubMed, Medline and Scopus databases was conducted to identify studies of demeclocycline and doxycycline medicaments used in root canal therapy with articles in English only assessed. Exclusion criteria included articles on animal teeth, avulsed or reimplanted teeth, combinations with other antibiotics and pulp capping. Inclusion criteria involved articles on demeclocycline, doxycycline, ledermix, doxymix, doxypaste associated with root canal treatment, systemic effect demeclocycline and doxycycline, molecular chemistry of tetracycline antibiotics including (stability, oxidation, hydrolysis, degradation, discolouration) and laboratory studies of tetracycline staining in dental contexts were assessed in detail for the methodology used.

2.Mechanisms of tetracycline-induced discolouration

2.1 Effect of Light

Light accelerates the degradation of tetracyclines, including demeclocycline, when moisture and oxygen are present (20-24). The effect of light on tetracyclines and their derivatives is a form of photodegradation, driven when these compounds are irradiated with wavelengths that are strongly absorbed (25). Tetracyclines can absorb light in the visible and ultraviolet regions of the spectrum, which leads to the generation of the free radicals responsible for their degradation (26). Having a yellow colour, tetracyclines absorb violet and blue light particularly strongly. Consequently, these shorter wavelengths of light are more potent in causing photodegradation than longer wavelengths of light, such as those in the green or red regions (27). Comparisons of the effects of various types of light on demeclocycline, doxycycline, and tetracycline have shown that blue light has the greatest effect, followed by white light, whereas little change occurs with green or red light (27). The greater photon energy of the shorter wavelengths of violet and blue light is the reason why tetracyclines (and some other medicines) are stored in dark brown bottles, which filter these out (28).

The effect of light on tetracyclines can occur from exposure to artificial light as well as to sunlight. Demeclocycline hydrochloride can undergo photodegradation from extended exposure to the light from fluorescent lamps (29). Likewise, light exposure of the aqueous solutions of oxytetracycline also enhances its degradation three times faster than when the solutions are stored in darkness (30).

The effect of light on tetracycline stains within a tooth can be complex. For example, in one study, two parts of an extracted tooth with tetracycline discolouration were treated differently; one of the split halves was kept in the dark, whereas the other was exposed to light. The pigmented dentine and enamel darkened further on exposure to light, whereas the other half did not (31).

Tetracycline antibiotics bind strongly and rapidly to hydroxyapatite, which explains why the discolouration of teeth caused by tetracycline antibiotics appears to be permanent (32). The complex of bound tetracycline undergoes oxidation to become a new tetracycline molecule, known as 4α, 12α-anydro-4-oxo-4-dedimethylaminotetracycline (AODTC), acquiring an oxygen atom into its overall structure. This changes the color of the material from yellow to a red-purple colour (33).

A total of five in vitro studies using human teeth (4, 15-18) stored teeth in the dark and investigated the effects of light on discol-

oration from Ledermix paste. In three studies (15-17) there was no staining with the closed access cavities and the teeth stored with gauze soaked in water resulting in 100% humidity in a closed container. But in two other studies (4, 18) staining occurred within two weeks in the presence of open access cavities and 100% humidity. Moreover, taking samples kept in the dark and then exposing them to light caused the red-purple colour of AODTC to appear. This emphasizes the role of a photo-oxidation process driven by short wavelengths of light (3, 27).

This then raises the question of how much short-wavelength light teeth are likely to encounter, in a way that can interact with tetracycline medicaments placed within the root canal. The 470-nm visible blue wavelengths used to initiate polymerization of light-cured restorative materials affect tetracycline antibiotics differently, with demeclocycline being more reactive to light than doxycycline (27). The application of a dental curing light to a tooth could therefore contribute to the darkening of bound tetracycline compounds. Apart from the setting of a dental appointment, one must also consider ambient sunlight and artificial light, which can contain violet and blue light. Once light strikes the surface of teeth, some will be absorbed, some scattered, and some transmitted (34). The amount of scattering is inversely proportional to the wavelength of the light, meaning that violet and blue light will scatter the most and red light the least. Light that reaches the mouth by passing through the soft tissues of the lips and skin will have little blue light because this will be absorbed as it passes through blood vessels. This is because hemoglobin (both oxygenated and not) is a strong absorber of violet and blue light, whereas red light penetrates well through soft tissues (35, 36). To reach the crowns of teeth when the lips are not apart, light has to travel through the lips, which may be up to 30-mm thick (37). Beyond that, light that reaches the attached gingiva will be scattered by the dense collagen fiber network and the irregular stippled surface (38). The thickness of the attached gingiva varies: 3–4 mm for incisors and canines, 1.5–2.0 mm for premolars, and 2-3 mm for molars (39). When considering the light that passes through the gingivae and reaches the bone, attenuation within the bone occurs. The mean thickness of the labial alveolar bone overlying maxillary anterior teeth is approximately 1.0 mm and then increases to 2.0–5.0 mm on posterior teeth depending on their location in the arch (40, 41). Any light that reaches the crown or root of a tooth will be subject to scattering at the enamel or root surface as well as at various internal boundaries, such as the dentino-enamel junction. Scattering of blue light will be greater than that for longer wavelengths of light (42, 43). The amount of blue light that is transmitted per mm of enamel is 40% when in a dry state but only 20% when the enamel is hydrated. There is further attenuation of blue light in dentine, with only 10% or less of blue light transmitted through per mm of dentine (34, 44, 45).

Taken together, these considerations mean that little ambient sunlight or artificial light is likely to reach the root canal of a tooth to interact there with tetracycline medicaments. However, there are potential issues when intense curing lights are used on anterior teeth, e.g., for curing a resin composite in the access form of an anterior tooth, although this is a one-off event of short duration.

2.2 Oxidation

Oxidation is a very common pathway of drug degradation, and this process affects tetracyclines. The photochemical (light-induced) degradation of medicines almost always takes place in the presence of oxygen, being faster when oxygen levels are higher. Oxidation reactions that are induced by light (photo-oxidations) involve the activation of ground state molecular oxygen via energy transfer (a type II photo-process) to produce singlet molecular oxygen. This form of oxygen is more reactive than the normal ground state form and can oxidize substrates as well as form reactive oxygen species (a type-1 photo-process), such as the superoxide anion radical (O2–), hydrogen peroxide (H2O2), and hydroxyl free radicals (HO.). Oxidation can also occur when molecular oxygen is incorporated into a molecule, as in photo-oxygenation (46-48). In the case of tetracyclines, the phenolic core, dimethylamine branch, and conjugated double bonds are all prone to attacks by oxidation. Upon exposure to light, tetracyclines may undergo type I or type II degradation as well as direct oxygen incorporation (46-49). As already mentioned, for the latter, photo-oxidation results in the formation of AODTC, with one atom of oxygen incorporated in the oxidized tetracycline molecule (33).

Early investigations have revealed that the rate of photo-oxidation of tetracyclines in aqueous solutions is proportional to light intensity and is oxygen-dependent (20, 33, 49). More rapid breakdown occurs with increasing concentrations of dissolved oxygen because this is the source of singlet oxygen and reactive oxygen species. More singlet oxygen is generated with reducing pH, and more hydrogen peroxide with increasing pH (47-49). The dependency on oxygen is well illustrated by an experiment in which the complete absence of oxygen caused tetracycline antibiotics in solution that were irradiated with short-wavelength violet and blue light to degrade at only one third the rate than they did in normal room air, when exposed to the same intense light (23). The presence of oxygen likewise influences the extent of radicals generated by tetracyclines (21)

The percentage of oxygen in air is approximately 21% but falls to 13.2% in arterial blood and 6.4% in bone (50). Because blood supply is absent in a root canal, the amount of oxygen is very low. Consequently, oxidative degradation of tetracyclines will not occur to any great extent as long as the conditions remain anaerobic. In contrast, if the access cavity restoration is leaking or absent, oxygen as well as moisture will enter. The same could occur through caries, cracks, or failing restorations. Because many teeth will have one or more of these latter causative factors, it is imperative to remove these before commencing endodontic treatment (51).

2.3 Moisture

The presence of moisture is another key factor in the breakdown of tetracycline antibiotics because it allows their hydrolysis. This form of chemical decomposition can occur in the presence or absence of light (46). First-generation tetracyclines (such as oxytetracycline, chlorotetracycline, and tetracycline HCl) are known to be unstable in solution (regardless of pH) during storage. Demeclocycline has slightly improved acid stability than these and undergoes slower degradation

in solution. Second-generation (such as doxycycline and minocycline) and third-generation tetracyclines (tigecycline) are very stable in solution at an acidic pH. Moreover, they do not degrade under alkaline conditions (such as would occur with the use of calcium hydroxide) to their iso-form (51, 52).

The presence of moisture enhances the photo-oxidation of tetracyclines. This has been shown in an experiment where hydroxyapatite was added to tetracycline in a buffer solution of pH 7.4. When exposed to short-wavelength violet and blue light, the tetracycline in solution underwent photo-oxidation to form AODTC. This occurred 30 times faster than when the same light source was used to expose dry samples of bound tetracycline (32). Regarding the level of moisture in teeth, vital dentine has a nominal water content of 24% by volume, of which some twothirds resides in dentine tubules, and the remainder is bound up in the dentine matrix. Once teeth become non-vital, the water content reduces, and there is little free water remaining in the canal to interact with tetracyclines (53). It is important to note that in most in vitro studies of discoloration by tetracyclines, roots or teeth have been stored in 90%–100% humidity. This provides moisture as well as dissolved oxygen. The increased moisture content will facilitate the diffusion of tetracycline molecules into the dentinal tubules. It will also provide the key factors that promote tetracycline degradation, namely, water and oxygen. Thus, future studies should be undertaken using lower levels of humidity to provide a more realistic setting.

2.4 Other factors affecting discolouration

Sodium hypochloride (NaOCl) irrigant is used widely in endodontics. This can oxidize tetracyclines and promote the formation of AODTC. However, the resulting red-purple staining of dentine will not occur when oxidation is prevented by the use of anti-oxidants, such as 10% ascorbic acid (3).

The presence of particular metal ions can affect the stability of tetracyclines. Ferric ions (derived from blood degradation) have been shown to enhance the degradation of tetracycline HCl, oxytetracycline, and chlorotetracycline in aqueous solutions even when no light was present (54). When bismuth ions are present in teeth (e.g., following the use of MTA cement or AH 26), these interact with tetracycline antibiotics, causing greater discoloration following exposure to light (27). Calcium ions have been shown to enhance the photo-degradation of tetracycline HCl under simulated sunlight (55), a point of particular relevance given the calcium ion content of saliva, in the situation of coronal leakage. Finally, simply cleaning the access cavity repeatedly with cotton wool pellets soaked in alcohol until there are no traces seen on the pellets will ensure that any excess paste left behind has been minimized.

Bringing together several strands from the foregoing discussion, a fluid-proof coronal restoration will prevent the entry of moisture and oxygen, two of three main contributors to tetracycline degradation. This point applies particularly to interim restorations during root canal treatment, which aim to help maintain a bacteria-free and fluid-free environment within the root canal system (51). Hence, clinicians should carefully consider how temporary restorations are placed and the materials that should be used.

DISCUSSION

In evidence-based dentistry, the goal is to integrate the best research with the best available clinical evidence (56). In the case of tetracycline staining from endodontic medicaments, there is no high-level evidence from systematic reviews, meta-analyses, or double-blind randomized control trials. The evidence that demeclocycline in Ledermix paste can cause tooth discoloration comes from six laboratory studies that use the same methodology and do not replicate all elements of the clinical setting (discussed below). Only two narrative reviews (57, 58) mention discolouration from Ledermix paste as a small section of their overall content. Both reviews cite earlier in vitro studies from the same authors (15, 16, 59). Thus, the evidence supporting the effect is limited and weak and runs counter to the clinical setting, where patients are followed over several years and which provides a different perspective.

Figures 1 and 2 are cases that demonstrate that demeclocycline and doxycycline-containing medicaments do not necessarily cause tooth discolouration even when left in place for extended periods of time in root canals with comprehensive interim restorations. These cases are not unusual in the authors' clinical practices. The three people in the case reports

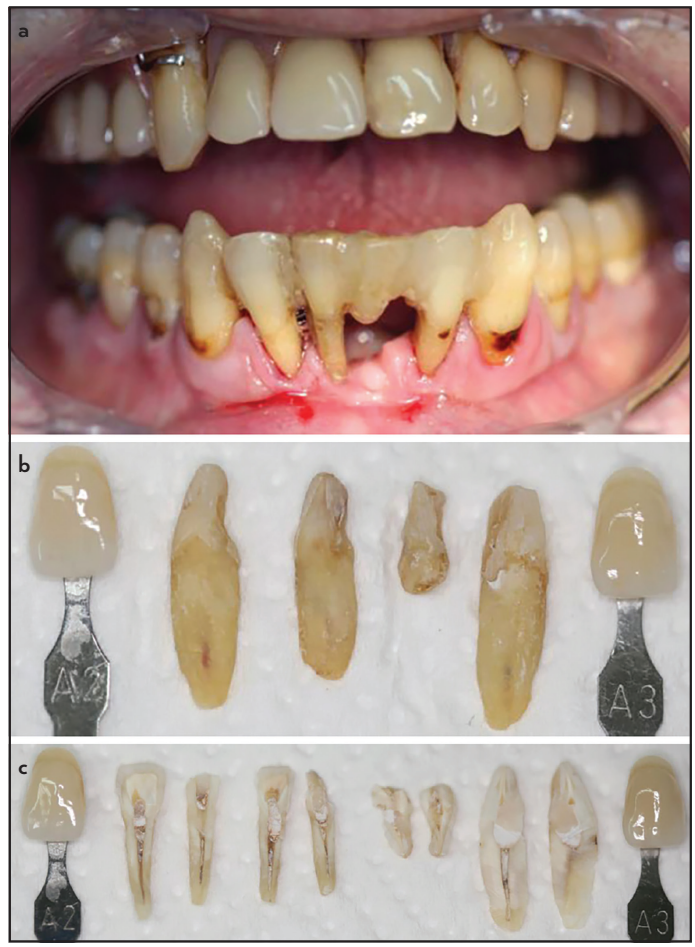

Figure 1. (a-c) Ledermix paste left in place for 5 months Teeth in the patient's mouth prior to extraction (a), The teeth immediately after extraction (b), and Longitudinal cross-sections of the teeth show no discoloration of the teeth (A2 and A3 Lumin Vacuum shade tabs serve as reference points) (c)

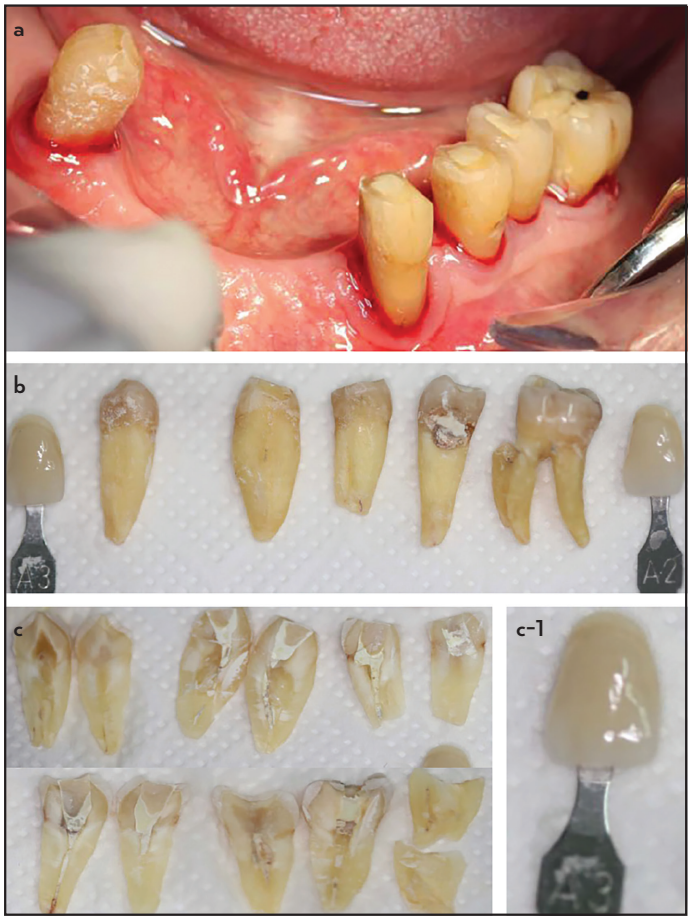

Figure 2. (a-c) Doxycycline paste left in place for 4 months Teeth in the patient's mouth prior to extraction (a), The teeth immediately after extraction. The first tooth to the left in the photos had no dressing placed and is useful for a baseline reference (b), and Longitudinal cross-sections of the teeth show no discoloration after 4 months with doxycycline dressing (A3 Lumin Vacuum tab as reference) (c)

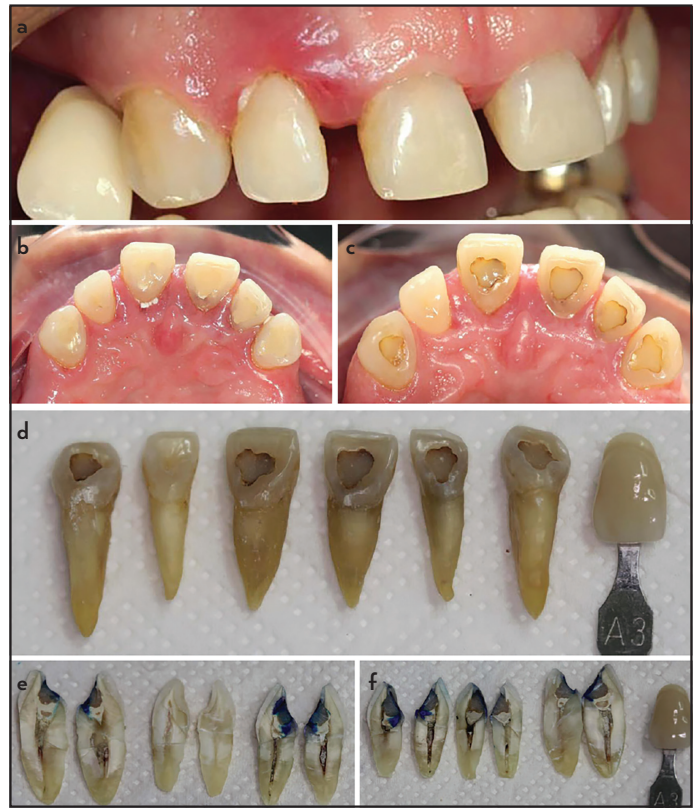

Figure 3. (a-f) Ledermix paste left in place for 3 months Teeth in the patient's mouth prior to extraction (A2 Lumin Vacuum shade tab as reference in the top left picture) (a), Palatal view of the access cavity on day 1 after the teeth were first dressed with Ledermix paste. The second tooth to the left (12) in the photos had no dressing placed and is useful for a baseline reference (b), Palatal view of the access cavity at 3 months after the teeth were dressed with Ledermix paste. The second tooth to the left (12) in the photos had no dressing placed and is useful for a baseline reference (c), The teeth immediately after extraction (d), Longitudinal cross-sections of the teeth (13, 12, 11) with methylene blue in the access cavity (e), and Longitudinal cross-sections of the teeth (21, 22, 23) with methylene blue in the access cavity (A3 Lumin Vacuum tab as reference) (f)

originally presented to the general dental practice of one author (BA) with various symptoms. The teeth were in very poor condition due to decay, periodontal disease, pulpal involvement and a decision to extract the teeth was decided upon after careful consideration of all the options. The three people had knowledge of the proposed treatment outlined in this article, gave their informed consent prior for treatment carried out and for photographs of the extracted teeth, had regular follow up on a weekly basis and access to emergency care if required. There was no post-operative pain reported in the three cases reports. Partial or full dentures were inserted immediately after the teeth were extracted.

Figure 3 shows the effect of a poorly adapted temporary access cavity restoration on the discolouration of roots. In the cases shown in Figures 1 and 3, Ledermix paste was left in the teeth for 5 months and 3 months, respectively. In the teeth shown in Figure 2, the canals were medicated with a paste containing doxycycline hyclate (Doxymix-Ozdent, Sydney, Australia), which has a similar base to Ledermix paste (i.e., polyethylene glycol PEG 400/4000), for 4 months. In all three cases, the root canals were irrigated with 2% NaOCl and 17% EDTA, with EDTA as the final irrigant. After drying of the root canals using paper points, the pastes were placed into the root canals with a spiral root filler in a low-speed handpiece. Excess paste was removed from the access cavity with cotton wool pellets (Figures 1 and 2). The access cavities were filled with Cavit/Fuji II LC (GC Corporation, Tokyo, Japan) (Figures 1 and 2) or Cavit / Fuji 8 (GC Corporation, Tokyo, Japan) (Figure 3). No cotton wool was left in the access cavity to maximize the thickness of the temporary filling material. Two thick coats of Ledermix paste were placed in the access cavity, and the excess paste was not removed prior to temporization with Cavit and Fuji 8 (Figure 3). This resulted in poor adaptation of the temporary filling to the wall of the access cavity.

No discolouration of the teeth is evident in Figures 1 and 2 despite the presence of significant gingival recession and the teeth are placed in the front of the mouth, which would have enhanced exposure of the roots and crowns of the teeth to natural light. In contrast, definitive greying of the treated radicular roots can be seen in Figure 3. The addition of methylene blue to the access cavity, after the teeth were extracted,

highlights the areas where the restoration allowed the ingress of fluid and bacteria. Taken together, these cases illustrate the point that discoloration from tetracycline-containing medicaments should not be expected to occur if the temporary filling in the access cavity prevents the ingress of both moisture and oxygen.

In the six in vitro studies (4, 14-18) that have described discolouration with demeclocycline and doxycycline medicaments, the key factors in their methodology that are operating are as follows. First, the roots were stored in dilute NaOCl initially; second, during endodontic treatment, the root canals were irrigated with NaOCl, some of which is likely to have spilled over onto the outer root surface. This results in excessive Na-OCl, which oxidizes the tetracycline medicament. Third, access cavities were left open, allowing oxidation and hydrolysis of the antibiotic. An alternative regime would be to store the extracted teeth in 0.1% thymol solution (with or without gamma irradiation), use a high-speed diamond bur or ultrasonic scaler (both with water spray) to remove soft tissue remnants from the roots, confine the irrigant solutions to the canals and not allow them to spill over onto the root surfaces (e.g., by using high-speed suction or Endovac irrigation), keep the prepared teeth/roots in containers with distilled water for 2 weeks in ambient room light to minimize any irrigant contamination, clean the root surfaces with ethanol after any medicaments have been placed, fill the access cavities with a sandwich restoration of Cavit/resin-modified glass ionomer cement, and then keep the teeth in an atmosphere with physiological oxygen levels (50) and low moisture (53) in a model that replicates the presence of bone and overlying soft tissues instead of placing teeth in clear plastic containers.

Knowing the basis of staining, there are several materials-based approaches that should be considered to reduce this problem; these are not mutually exclusive. The first strategy would be to alter the composition of endodontic pastes, replacing demeclocyline hydrochloride with doxycycline hyclate but keeping other components of the paste the same (4). A second approach would be to include an appropriate antioxidant, such as ascorbic acid (vitamin C), into the paste to reduce the degradation of tetracycline (60). A third approach would be to rinse the root canals with a 10% ascorbic acid solution prior to placing tetracycline-based medicaments (3). Finally and most importantly, a non-leaking interim restoration is needed to prevent the ingress of moisture and oxygen.

CONCLUSION

Tetracyclines are a unique class of antibiotics because they exert antibacterial actions and also have additional effects that are anti-inflammatory, anti-resorptive, and substantive within the root canal and long-acting in the surrounding tissue. Several factors that affect tetracyclines can cause their breakdown and the subsequent staining of teeth. These factors are interrelated, making the discolouration process multi-factorial in its etiology. Many of these factors have not been considered in previous research on discolouration associated with endodontic medicaments such as Ledermix paste. Light in the presence of moisture and oxidation are the key factors that drive the discoloration process. Control of these can be achieved by the removal of all restorations, cracks, and caries prior to endodontic treatment; by having a sound interim restoration that will remain in place for the period of the root canal treatment; and by the removal of excess tetracycline paste from the access cavity by cleaning the access cavity repeatedly with cotton wool pellets soaked in alcohol until there are no traces seen on the pellets and a definitive final restoration of the tooth. Great care is needed when drawing any conclusions from previous in vitro studies that have used Ledermix paste because these have not replicated all aspects of the clinical situation. For future laboratory studies of endodontic medicaments, an improved methodology that more closely matches what happens clinically is proposed. Steps to prevent discolouration include the replacement of demeclocycline with doxycycline and the use of a 10% ascorbic acid rinse prior to applying tetracycline medicaments.

Disclosures

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Conflict of interest: The authors declare no direct conflicts of interest. Paul Abbott declares that he has acted on occasions as a Consultant to OzDent Pty Ltd., the manufacturers of Calmix, and the Australian suppliers of Ledermix paste. Basil Athanassiadis and Laurence Walsh have a licensing agreement with Ozdent Pty Ltd., the manufacturers of Calmix. OzDent Pty Ltd., had no role in the design of the study, in the collection, analyses, in the writing of the manuscript, or in the decision to publish the results.

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