Radiographic changes in endodontically treated teeth submitted to drowning and burial simulations: is it a useful tool in forensic investigation?

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KEYWORDS

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ABSTRACT

Dental radiographs, endodontic treatment and materials are a source of useful forensic data. The response of dental materials to death-related events are widely studied and provide forensic evidence for experts. This study aimed to analyze the radiographic images of endodontically treated teeth submitted to burial and drowning simulation, verifying its forensic feasibility, applicability and usefulness. Material and method: n=20 bovine incisor teeth were endodontically treated then divided into two groups: burial and drowning scenarios. Teeth were radiographed two times (before and after scenario) with an aluminium stepwedge, and optical density (OD) was assessed in each root third, in both radiographs, and then compared (ANOVA and Tukey test) for each scenario. Results: Burial scenario did not significantly alter radiopacity. As for the drowning scenario, there was no difference in radiopacity between the root thirds before the test. After drowning, the apical third demonstrated lower OD ($p<.05$) than the other two thirds. Comparing the OD before and after drowning, medium third presented lower and cervical third demonstrated higher means ($p<.05$) after drowning. Conclusion: We concluded that drowning conditions could alter the radiopacity of endodontically treated teeth, more specifically in the medium and cervical thirds. There is no evidence that this also occurs in burial situations. This has the potential to be useful in forensic casework as an initial sign of the type of ambient in which the body was supposedly exposed or set.

INTRODUCTION

The INTERPOL DVI Guide (2018) establishes that fingerprint analysis, dental information, and DNA (so called primary methods of identification) are self-sufficient in the identification process, and do not require further investigations when they establish an identity.[1] When death results from extreme situations, and human remains are severely destroyed or burned, forensic odontology is an essential forensic tool, as teeth are very resistant to hazardous conditions, such as high temperatures.[2]

Dental arches, despite having characteristics that may change throughout life, have a combination of traits that are reproducible and can be compared at any time, granting uniqueness through teeth positioning, anatomical features, treatment provided and pathology in every single person.[3] In this sense, not only clinical examinations but also dental radiographs play an important role in the identification process

by making feasible the comparison of those features at two anatomical levels of complexity: external and internal dental anatomy.[4]
Radiographs are frequently found in dental records, enabling ante-mortem (AM) and post-mortem (PM) comparisons, through the observation of dental anatomy, restorations and endodontic treatment, which are easy to detect radiographically.[3] Consequently, endodontic treatment is valuable for forensic odontology, given the extensive use of post-operative radiographs, thus providing important ante-mortem data.[5]
Although the prevalence of endodontic treatment varies considerably worldwide, usually due to the heterogeneity of populations and endodontic protocols,[6] in Brazil this number is relatively high[7] compared to other countries[8]. Understanding the response of dental tissues and dental materials submitted to hazardous conditions is significant to forensic science as it enhances the identification process.[9-10]
In this context, changes in endodontic materials submitted to hazardous conditions, such as high temperatures, was already studied by analyzing several parameters such as macroscopic,[11] microscopic,[12-13] and radiographic changes.[12,14] However, their effect when set to burial or drowning simulations has not yet been evaluated. The number of other studies in these scenarios (burial and drowning) is scarce.[15-16] These situations are reportedly real conditions in routine forensic odontology and criminal investigations[2,17].
This research aimed to analyze the radiographic images of endodontic treatment, in teeth submitted to burial and drowning simulation, verifying its forensic feasibility, applicability and usefulness.

MATERIAL AND METHODS

For this study, inclusion criteria were bovine incisors with a single root canal – the exclusion criteria were teeth with cracks or fractures, or without complete root apex formation. The final sample number was (n=20) teeth.
The teeth were rinsed in running water, and using periodontal curettes, the tissue remnants adhering to the dental surface were removed. They were then rehydrated in 0.9% saline solution and then stored in a glass vessel with distilled water under refrigeration until use. They were then sectioned perpendicularly to its long axis, above the amelo-cemental junction, separating each tooth in a coronary and a root portion, standardizing the length of the specimens in 30 mm (Fig. 1). In order to establish a default sample size, crown sectioning was done to facilitate the insertion of the K-type files, which had a maximum length of 31 mm and could become lodged at incisal edges, due to the extensive length of bovine teeth. The crown portions were then discarded.
The root portions were then submitted to endodontic treatment using manual instrumentation with K-type files (Dentsply Maillefer Indústria e Comércio Ltda., Petrópolis, Brazil) and irrigation with 0.5 ml of 1% sodium hypochlorite solution (ASFER Indústria Química Ltda, São Caetano do Sul, Brazil). The root canals were filled with gutta-percha cones (Dentsply Maillefer Indústria e Comércio Ltda., Petrópolis, Brazil) and zinc oxide and eugenol endodontic cement (Endofill - Dentsply Maillefer Indústria e Comércio Ltda, Petrópolis, Brazil), being obturated by the cold lateral compaction technique[18].
The sealing of the remnant coronary portion of the root canal was made by composite restoration, using 37% phosphoric acid gel (Maquira Indústria de Odontológicos, Maringá, Brazil) for 15 seconds in dentine in etching phase, then two layers of Adper Single Bond 2 adhesive system (3M ESPE Dental Products, Sumaré, Brazil), and finally Filtek Z250 XT micro-hybrid composite resin (3M ESPE Dental Products, Sumaré, Brazil), photoactivated (FLASH Lite 1401, Vigodent Coltene, Belo Horizonte, Brazil) for 20 seconds. The restoration was then polished and finished out at high speed and under refrigeration with 30-blade multilayer drill bit FF 9714 bur (JET Carbide Burs). Samples were randomly divided into two groups (n=10); each one submitted to a simulation of the two environmental conditions.

Environmental conditions

The burial condition was simulated, putting the teeth into a tissue bag filled with soil and placed inside an excavation with a depth of 1.0 m, and covered by the same land, in an area delineated for research. The drowning condition was simulated, putting the teeth in a pouch similar to that used for the burial scenario, placed in a cage immersed in a natural lake. These conditions were simulated for a time-lapse of 90 days. All the bags and pouches had little perforations over their surface, which guaranteed that teeth had full contact with the environment, as well as keeping them from drifting away in the water or losing them in the soil.

Acquisition of radiographic images

Each sample was submitted to two radiographic exposures (Spectro 70X, Dabi Atlante Indústrias Médico Odontológicas Ltda., Ribeirão Preto, SP, Brazil, 70kVp, 8mA, focus/film distance of 20 cm, and exposure time of 0.28s). One exposure was made prior to drowning and burial submission, and the second exposure was made 90 days after being exposed continually to these scenarios.

For the radiographic procedure, the teeth were placed on radiographic films in a standardized way and with an aluminum stepwedge measuring 10 x 32 mm, and scaled in 8 steps, with incremental thicknesses of 2.0, varying from 2.0–16.0 mm in thickness. The purpose of this stepwedge placement was to simulate the densities of the soft and hard tissue structures of the oral cavity, by producing a scale image of radiopacity nuances resulting from radiation exposure and film processing; for assessment of radiographic quality; and to observe the homogeneity of the exposures.[19]

Sample analysis

The optical density (OD) values of the endodontic materials were assessed in the radiographs, in each tooth root third, (cervical, middle and apical) by a photodensitometer (MRA Indústria e Equipamentos Eletrônicos Ltda, Ribeirão Preto, SP, Brazil) with a collimated light beam regulated to a diameter of 1mm. OD reading was made in triplets for each dental third of each radiograph. The stepwedge "steps" had their OD also individually measured. Then, the OD mean values of those triplets were converted to equivalent millimetres of aluminum (mmAlEq), according to Guerreiro-Tanomaru et al. (2009),[20] for statistical analysis. OD mean values of root thirds and aluminum stepwedge were compared. Values below 3mm of mmAlEq were considered as unacceptable or indistinguishable from radiographic imaging.

Mean values of mmAlEq, before and after the environmental conditions, in the different root thirds (cervical, middle and apical) were analyzed by 2-Way ANOVA, and Tukey test, at the significance level of α = 5%.

RESULTS

The mean values for OD readings, on the different thirds, submitted to different conditions, can be seen in Figs. 1 and 2. Before the scenarios, the middle third always presented the highest radiopacity (mmAlEq = 15.55 in both), while the lowest values were observed in the apical third (11.88 and 11.53 for drowning and burial, respectively).

Figure 1. Mean values of mmAlEq (Equivalent Aluminium Milimeters), in the different root thirds, before and after drowning of the samples

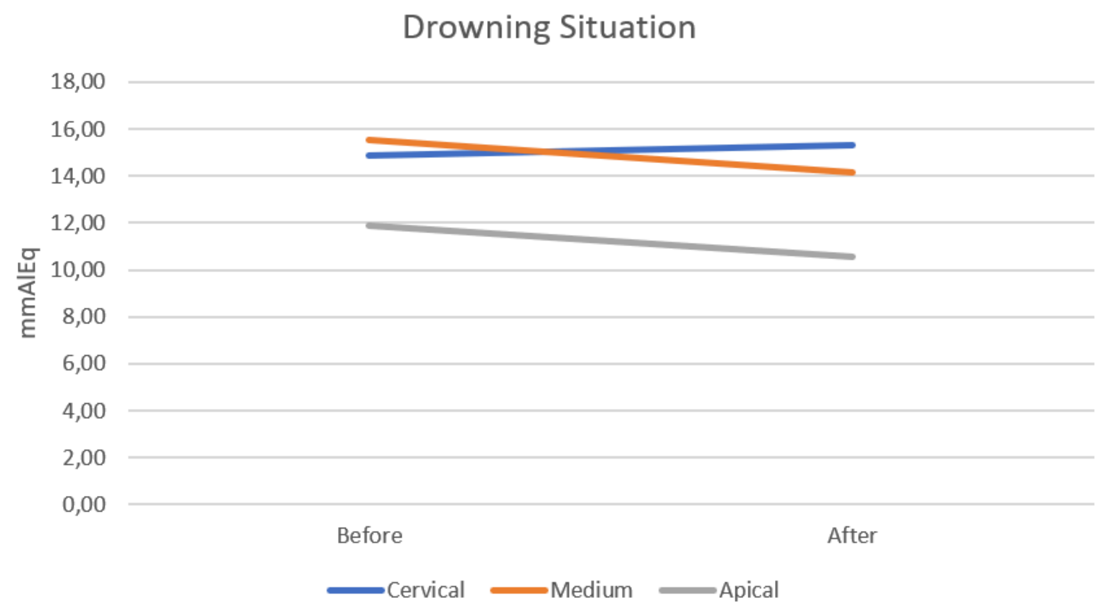
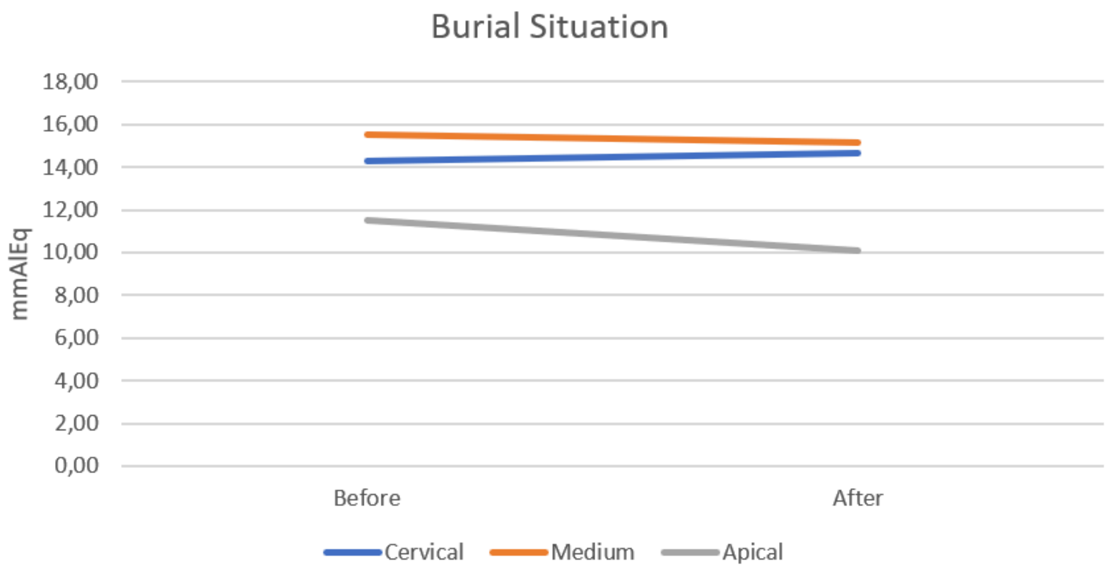


Figure 2. Mean values of mmAlEq (Equivalent Aluminium Milimeters), in the different root thirds, before and after burial of the samples



The groups means comparison (2-way ANOVA, Tukey, p<.05) are shown in Tables 1 and 2. Lower radiopacity (p<.05) was observed in the apical third of the obturation, in comparison with the other two analyzed thirds, before and after burial conditions. Also, no evidence was found that burial alters the radiopacity of the endodontic materials.

Table 1. Mean Values, in mmAlEq, in the different root thirds, before and after burial. Different letters, upper case in column and lower case in line, indicate statistically significant difference (p <0.05).

Burial			
Root Third	Cervical	Medium	Apical
Before	14,27 aA	15,55 aA	11,53 bA
After	14,68 aA	15,13 aA	10,06 bA

Table 2. Mean Values, in mmAlEq, in the different root thirds, before and after drowning. Different letters, upper case in column and lower case in line, indicate statistically significant difference (p <0.05)

Drowning			
Root Third	Cervical	Medium	Apical
Before	14,84 aB	15,55 aA	11,88 aA
After	15,34 aA	14,14 aB	10,53 bA

Prior to submission to the drowning scenario, there was no difference in radiopacity between the root thirds. In the aftermath of this condition, the apical third demonstrated lower OD (p<0.05) than the other two thirds. When comparing the OD before and after the drowning simulation, the medium and the cervical thirds presented lower higher means (p<0.05), respectively.

DISCUSSION

Understanding the influence of catastrophic or adverse events on different dental materials is vital, since some disasters can result in the dispersal of victims in environments with extreme temperatures, and other situations.[21] With this in mind, some studies observed alterations in several restorative materials subjected to such extreme conditions, as the staining and colour stability of different composites submitted to high[22] or low[23] temperatures.

Endodontic treatment is a valuable source of forensic data, because of accompanying radiographs which are suitable for comparison.[5] This analysis is often made as a qualitative comparison between suspects' and the unknown individual's ante-mortem (AM) and post-mortem (PM) information, regarding morphological aspects of the teeth, treatment, obturation shape, and materials used.[24] When endodontically treated teeth are involved, this examination rarely involves an analytical and quantitative approach.

There are several reports in the literature[11-14,25] about endodontic materials submitted to forensic conditions. However, the majority are about the influence of high temperatures. In this study, we analyzed the influence of drowning and burial scenarios in the radiopacity of endodontic materials, using optical densitometry.

Endodontic interventions are composed of several stages, such as: disinfection, modelling, and sealing of the root canals. In the final stage, sealers are employed to obturate the prepared canals, generally using materials that have satisfactory radiopacity, biocompatibility, solubility, and thermoplasticity.[26] Regardless of the technique used, gutta-percha cones are traditionally used for root canal sealing. This material is composed mainly of zinc oxide, gutta-percha itself, waxes, resins, and heavy metal radiopacifiers. Its chemical composition does not vary significantly between different manufacturers.[27]

Nevertheless, endodontic cement can be based on different components, like calcium hydroxide, zinc oxide with eugenol, glass ionomer, silicone, resin polymers, and calcium silicate.[28] In our work, we used the combination of gutta-percha cones added to zinc oxide and eugenol-based cement.

The lowest values of radiopacity were found in the apical third of the root canals, in both groups, before and after drowning and burial. Radiopacity, in general, varies according to the cement thickness around the filling material.[29] Thus, as the apical third usually has a smaller taper and a greater narrowing before the endodontic preparation, smaller amounts of material and cement are found.

In the group submitted to drowning, after the 90 day time-lapse, it was observed lower means of radiopacity in the middle third and a higher value for the cervical third. This finding can be attributed to cement solubility, as eugenol and zinc oxide-based cement have a higher solubility among cement types due to the continuous loss of eugenol from its matrix, diminishing mass and causing the contraction of the material.[28,30] Changes in the plasticity of gutta-percha could also explain this response, despite being considered an innocuous material in water, and low temperatures.[31]

Radiopacity lowering also occurs at high temperatures, as observed in other studies.[12,14] However, in these cases, the endodontic obturation displays unique patterns, macroscopic alterations, along with evident tissue destruction of the tooth remains.[11] By consequence, charred remains of endodontic materials are easily distinguished from those studied here.

Furthermore, we observed no significant radiopacity changes between before and after the burial process. The conservation and stability of dental tissues during burial has been reported by Menon et al. (2011).[15] Despite this, further studies with human teeth are necessary to verify the present results. It is necessary to remark that our study was conducted with bovine teeth, which, despite being routinely used in dental research, can behave differently than human teeth, depending on the analysis done.

The human body has known responses towards death, which involve morphological changes (colour, shape, size, integrity) due to taphonomic events such as decomposition, putrefaction, mummification and skeletalization, which are broadly studied in forensic medicine, given their importance in establishing the *causa mortis*.[32] In forensic odontology, this is no different, and these thanatological findings must be carefully studied and registered as they can potentially help in human identification.[33]

Soil compounds and characteristics are well known to exert influence in these taphonomic changes.[34,37,38] This microenvironment usually triggers or delays the decomposition rate in burial situations, produce signals and leave traces that indicate the type and conditions of the burial site's soil. [34] Similarly, bodies submerged in water during or after death, in a drowning event, also have typical thanatological outcomes, such as the still debatable pink teeth, as a possible dental alteration related to wet or moist environments.[35]

It is also well known that soils, in general, have a significant presence of acids in their composition, originating from several sources.[36] When a soil environment has a high concentration of acids, it may become acidic, and buried bone structures are more prone to changes caused by this condition, as observed by Howes et al. (2012).[37] Highly acidic soils can rapidly decompose bone by dissolving its hydroxyapatite (inorganic matrix)[38], the same substance that composes the majority of dental enamel.

Mazza et al. (2005)[39] observed alterations in dental structures immersed in different types of acid with time and concluded that even when teeth are in direct and complete contact with acid, it is still possible to analyze and recognize its structures until the advanced stages of degradation. A remarkable observation in this study is that one sample tooth studied presented a residual structure after being submitted to acid, which later was identified as gutta-percha bulk. Subsequently, this material was divided into two fragments and immersed again in two different acids, enduring dissolution still, for over 50 hours. As it was already pointed out before, gutta-percha is a stable and firm material that can help immensely in identification processes, which reinforces the importance of endodontic materials in forensic cases.

Although not case-specific nor pathognomonic, all these signs and outcomes discussed here can give initial hints and suggestions to the forensic expert, commencing the investigation of the events surrounding death. Dental and endodontic materials are liable to these mentioned environmental influences. The findings of the present study are relevant, as they indicate that radiographic alterations in the radiopacity of endodontic materials are significant in the drowning scenario. Changes also seems to occur in the burial scenario, but these were found not to be significant, and further research should be conducted.

Therefore, our results may represent an outset forensic finding, specifically in those cases where the cadaver was merged into water, set or left in an aquatic environment. Radiopacity changes are easily detectable with proper equipment and could indicate different conditions to which the body has been exposed, especially in those cases where morphological changes had still not happened nor are visible to the human eye.

Caution must be used when conclusions are drawn from the results of this study. It did not intend to link its results with the certainty of any cause of death, nor to indicate an aquatic submersion of a particular victim during death. More scientifically robust findings must be combined to establish a better conclusion, with larger samples and human teeth, in conjunction with real case reports. However, our results are important, since similar research has not yet been conducted, thus opening paths for researchers to findings related to routine forensic cases.

CONCLUSIONS

Drowning conditions can alter the radiopacity of endodontically treated teeth, specifically in the medium and cervical thirds. There is no evidence that this also occurs in burial situations. These findings have the potential to be useful in forensic cases as an initial sign or suggestion of the type of environment that the body was exposed or set. However, they should not link directly to the cause of death or lead to a case solution, as alternative scientific techniques should be conducted.

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