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Application of forensic luminol for blood detection in endodontic files

Aplicação do luminol forense para detecção de sangue em limas endodônticas

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Resumo

Introdução: Sangue é um material biológico com alto potencial de transmissão de infecção em ambientes odontológicos, incluindo herpes simples, hepatites e AIDS. **Objetivo:** Investigar a eficácia do luminol em detector sangue em limas endodônticas antes e após o processo de esterilização. **Material e método:** Luminol foi utilizado para investigar a presença ou ausência de vestígios tecido sanguíneo em 50 limas endodônticas, visíveis ou não à olho nu, após a realização do tratamento endodôntico e após o processo de limpeza/esterilização. Os resultados obtidos foram tabulados e analisados estatisticamente utilizando o teste de Friedman com nível de significância de 5% ($p<0,05$). **Resultado:** A olho nú, foi observado que 31/50 limas não apresentaram vestígios de sangue, 8/50 apresentaram uma leve presença de sangue e 11/50 apresentaram uma presença considerável de sangue após o tratamento endodôntico. Após a utilização do luminol, entretanto, 16/50 limas endodônticas não apresentaram vestígios de sangue, 19/50 apresentaram uma leve presença de sangue e 15/50 apresentaram uma presença considerável de sangue. Após o processo de limpeza e esterilização não foi detectado sangue nas limas endodônticas. **Conclusão:** A solução de luminol é efetiva na detecção de tecido sanguíneo em limas endodônticas, validando o processo de limpeza/esterilização.

Descritores: Lima endodôntica; terapia endodôntica; endodontia; controle de infecção.

Abstract

Introduction: Blood is a biological material with high potential of infectious transmission in dental environments, including herpes simplex, hepatitis and AIDS. **Aim:** To investigate the efficacy of luminol in detecting blood in endodontic files before and after the sterilization process. **Material and method:** Luminol was used to investigate the presence or absence of traces of blood tissue in 50 endodontic files, visible to naked eye or not, after performing endodontic treatment and after the cleaning/sterilization process. The results obtained were tabulated and statistically analyzed by using the Friedman's test at a significance level of 5% ($p<0.05$). **Result:** By naked eye, it was found that 31/50 files showed no trace of blood, 8/50 showed a slight presence of blood and 11/50 showed a considerable presence of blood after endodontic treatment. After the use of luminol, however, 16/50 endodontic files showed no trace of blood, 19/50 showed a slight presence of blood and 15/50 showed a considerable presence of blood. After the cleaning and sterilization process, no blood was detected in the files. **Conclusion:** It was concluded that the luminol solution is effective in detecting blood tissue in endodontic files as well as in validating the cleaning/sterilization process.

Descriptors: Endodontic file; endodontic therapy; endodontics; infection control.

INTRODUCTION

Dental patients and dental healthcare workers may be exposed to a variety of microorganisms via blood or oral or respiratory secretions[1]. Cross-infection control will always be an important area of concern as new and emerging pathogens have been frequently isolated and drug resistance has been increasing as well. Moreover, this forms an important part of practice for all healthcare professionals and remains one of the most cost-effective medical interventions available[2].

Saliva and blood are biological materials with high potential of infectious transmission in dental environments. Major infectious diseases related to the dental surgery practice are herpes simplex,

hepatitis, and AIDS. Infections caused by viruses are the most severe and of most concern when contracted[3,4].

Dental care professionals are at high risk of cross-infection while treating patients[4,5]. The practitioners should adopt security measures for performing the care of the patients as they were all carriers of microorganisms with potential to cause infectious disease[6]. Currently, several methods of disinfection and sterilization are used to ensure the maintenance of the aseptic chain, including disinfectants such as glutaraldehyde, formaldehyde, alcohols, iodine and phenol synthetic[7].

It is extremely desirable and convenient to have secure methods for reliably detecting traces of substances in liquids, especially body fluids, since the presence of a substance at concentrations as low as 10^{-11} M may be potentially pathogenic[8].

The classical methods for detecting substances in liquids are based on reaction mechanisms where either appearance of a product or disappearance of a reagent can be measured. Greater importance is given to chemiluminescent compounds, which is capable of producing light when in favorable conditions. The chemiluminescence is a method based on certain substances with the characteristic to emit light when in presence of the compound to be analyzed. It has applications in forensics, diagnosis and quality control fields, being capable of detecting traces of compounds[8].

One example of a commonly used chemiluminescent substance in criminal investigations is luminol, which is capable of detecting blood stains hidden in suspicious locations, such as crime scenes. Its higher sensitivity to blood compared to other reagents and its non-destructive effect on DNA are some of the major advantages of lumimol[9].

In most luminol formulations, the 5-amino-2,3-dihydro-1,4-phthalazinedione is dissolved into an alkaline mixture with an oxidant agent[6]. In the presence of metal ions (Fe^{+2}) or metal complex, such as hemoglobin, the luminol oxidation produces a bright blue chemiluminescence[1]. The heme group in the blood, even at small concentrations, can act as a catalyst in the oxidation process of luminol in alkaline solution[10,11].

The luminol method is non-toxic and easy to use, being up to 20 times more sensitive to hemoglobin than other blood detection tests, including those using phenolphthalein (Kastle-Meyer test), leucomalachite green, benzidine reagent and fluorescein. It allows visualization of a 7-week trace of blood, which is invisible to naked eyes[12].

There are few studies on dentistry reporting the use of presumptive tests for detection of bloodstains[13], including the use of Kastle-Meyer reagent[14] and luminol[15]. However, the Kastle-Meyer reagent technique has more interferences and less sensibility than the luminol formulations*.

Endodontics deals with diseases of the dental pulp and periapical tissues, which are highly vascularized. During root canal treatment, endodontic files may come into contact with blood from these tissues and become carrier of viruses, such as that of hepatitis[16]. Therefore, the aim of this study was to investigate the efficacy of luminol in detecting blood in endodontic files before and after the sterilization process.

MATERIAL AND METHOD

This study has evaluated endodontic files used during endodontic treatment performed by final-year undergraduate students. The research was approved by the Human Volunteers Research and Ethics Committee, Dental School of Piracicaba – State University of Campinas - UNICAMP (protocol number 104/2013). All patients signed an informed consent form agreeing in participating of the research.

The 5-amino-2,3-dihydro-1,4-ftalazinadione used in this study was obtained according to a synthetic method developed by Lopes et al.[8] and the luminol reagent was prepared according to Weber's formulation[17]. Surfaces suspected to have occult blood were initially sprayed with a solution of luminol and sodium hydroxide contained in one vial, and then sprayed with a 3% hydrogen peroxide solution contained in a second vial.

Fifty endodontic files used during instrumentation of the apical third of root canals were included in this study. They were collected immediately after the treatment, without any washing and/or disinfecting process. Next, the files were individually stored in sterile glass vials with screw cap. Two examiners analyzed the endodontic files and confirmed the presence or absence of blood stains visible to naked eye. In a dark room, the file was covered with both solutions (luminol and hydrogen peroxide) in order to detect any trace of blood. If positive, the surface of the instrument becomes pigmented with fluorescent blue light, which is noticeable in an environment in the absence of light. If negative, no change occurs in the surface under these conditions.

The files were then washed and submitted to disinfection procedures and sterilization by autoclave according to bio-safety standards. Next, the endodontic files to be used in future clinical interventions were retested with the aid of luminol. In this phase, the endodontic files were collected from the autoclaved vials and again submitted to luminol test under the same light conditions previously described. It was expected that the compound would not detect blood in the dental instruments after the sterilization process.

The person who handled the contaminated material during the tests wore protective equipment, including gloves, mask, cap, goggles and disposable laboratory coat, to prevent contamination with microorganisms from blood, saliva, body fluids, secretions and excretions possibly present in the dental instruments analyzed.

Additional Luminol Tests

Meanwhile, in order to test the long-term effectiveness of luminol, a drop (50μL) was collect from one of the researchers. The same amount of blood (2.27μL) was placed on 22 glass slides to verify the effectiveness of the luminol solution after periods of 1, 3, 5, 7, 15, 30, 90, 180, 365, 540 and 731 days. This material was stored under room temperature in a closed container, with the necessary information for handling by authorized persons only.

Half of the slides prepared underwent successive luminol tests, whereas the other half was submitted to autoclave sterilization.

Furthermore, to investigate the effectiveness of luminol in contact with endodontic irrigant, the same amount of blood in different

glass slides was mixed with 2% chlorhexidine gel, 5.25% sodium hypochlorite and 17% EDTA. The slides were stored and analyzed after 24 hours.

Data Analysis

Assigned scores varying from 0 to 2 were used to identify the presence of blood:

Zero (0): unable to identify blood tissue by naked eye or in the presence of luminol, before and after the sterilization process.

One (1): slight presence of blood tissue was identified by naked eyes or in the presence of luminol, before and after the sterilization process.

Two (2): Considerable presence of blood tissue was identified by naked eyes or in the presence of luminol, before and after the sterilization process.

The results obtained were tabulated in a spreadsheet, analyzed by Biostat 5.0 statistical package (Belém, PA, Brazil), and submitted to the Friedman's test at significance level of 5% ($p<0.05$).

RESULT

Statistical analysis showed significant difference between the groups. In other words, there was statistical difference when comparing the scores (0, 1 and 2) as well as when comparing the detection of blood by naked eye and that by luminol before and after the sterilization process (Table 1).

Immediately after the endodontic treatment, blood could be detected in 19/50 by naked eye, however after the sterilization process its detection reduced by 100%. Luminol allowed detection of blood in 34/50 of the initial samples and in 0/50 after the sterilization process. Figure 1 shows the presence of blood in endodontic files detected by luminol.

Table 1. Detection of blood by naked eye and by luminol solution before and after the sterilization process

Score	Before sterilization		After sterilization	
	Naked eye	Luminol	Naked eye	Luminol
0	31[a]	16[c]	50[e]	50[e]
1	8[b]	19[d]	0[f]	0[f]
2	11[b]	15[c, d]	0[f]	0[f]

Different letters represent statistically significant difference ($p < 0.05$).

Luminol solution was able to detect traces of blood on the glass slides not only immediately after the application of blood on them, but also in the periods of 1, 3, 5, 7, 15, 30, 90, 180, 365, 540 and 730 days after storage under room conditions of temperature and humidity. Additionally, luminol was able to detect blood after 24 hours in contact with 2% chlorhexidine gel, 5.25% NaOCl and 17% EDTA.

DISCUSSION

Infection and cross-contamination control and these issues has become an essential part of their curricula and certainly cannot be underestimated by dental schools[18]. All individuals should be considered potentially contaminated and procedural protection against cross-infection should be adopted prior to dental procedures[4,6,19]. Direct contact between dental instruments and blood contaminated with HIV and/or HBV is a dangerous way of disease transmission[20,21].

HBV is a resistant virus, which may remain in dental instruments for over two weeks. It can be considered the highest risk of cross-infection in the dental office as an amount of 0.0001 ml of infected blood is sufficient for virus transmission[22]. The risk of contracting HBV during a piercing-cutting accident is higher compared to HIV[16,21]. Therefore, it is necessary to establish protocols for identification and elimination of transmitter agents of infectious disease. In this sense, luminol solution helps to detect traces of blood, increasing the efficiency of cleaning/sterilization processes of dental instruments.

The luminol used in the present study was developed by Brazilian researchers and is capable to detect invisible blood up to dilution of 1:100.000 without fluorescent lamps[23]. Another advantage is that this luminol does not destroy the existing DNA in blood stains, allowing its further analysis[8]. The main objective of the Brazilian luminol is to provide an alternative process for synthesis of hydrazides from dicarboxylic acids in safe and smooth reaction conditions, having an excellent yield.

The present research has showed that in the initial samples, where it was not possible to detect blood by naked eye (52% of cases), this rate decreased after the use of luminol (32% of cases). The amount of blood detected varied according to the case in which the endodontic file was collected, agreeing with the findings of Frégeau et al.[24] who reported that luminol could be used to detect the presence of very small quantities of blood or blood stains diluted up to a $1:10^6$ ratio.

We have also found that after 730 days of exposure to room temperature, luminol was still able to detect blood by emitting the blue light. The hemoglobin, while still within the body, remains

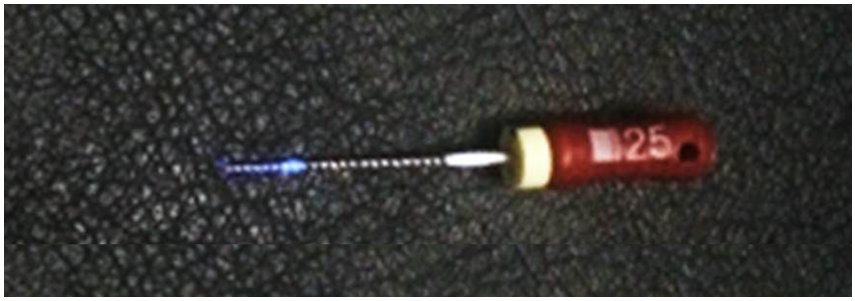

Figure 1. Presence of blood in endodontic files detected by Luminol.

protected by erythrocytes, which in turn possess enzymatic and non-enzymatic mechanisms to prevent its denaturation by maintaining the iron ions in the Fe^{2+} form. Despite the fact that blood suffers degradation when exposed to external environment, luminol chemiluminescence reaction remained effective, agreeing with the findings of Lopes et al.[8] who reported its effectiveness up to six years of the presence of blood contamination.

Our study has also shown that the substances used during chemomechanical preparation of root canals, such as chlorhexidine, NaOCl and EDTA, do not interfere with the luminol detection of traces of blood in the endodontic files. This finding is in accordance with that by Seashols et al.[25] who reported that the association of blood with EDTA does not cause major impact on its detection by luminol.

Sodium hypochlorite is one of the most important examples of interfering substances, since it is widely distributed throughout the domestic environment and is the most used disinfectant in hospitals. In addition, this substance can be used in an attempt to clean up a crime scene and remove blood evidence via its oxidation and physical elimination[10].

Hypochlorite is classified as a medium-strong oxidant with standard reduction potential ($E0$) of 0.841V and is capable of amplifying the chemiluminescence emission in luminol oxidation by hydrogen peroxide when both compounds are present in the reaction medium, including Grodsky's or Weber's formulations[10]. However, this is not a problem for a well-trained expert, who will be able to note the difference in the emitted wavelength by using the luminometer. The luminescence generated by the hypochlorite does not have the same wavelength (430 nm ± 3) compared to the one generated by the heme from the blood (455 ± 2 nm). The duration of light emission and the extinction form of both substances are different, since the luminescence produced by blood decreases with time, whereas that of hypochlorite dissipates immediately from the surface[26]. Furthermore, authors are improving the Webber's formulation in order to increase the intensity and time length of light emission and to eliminate false-positive reactions[27].

This research has demonstrated the importance of detecting blood in the instruments used in dentistry, since blood is a biological material with high potential of transmitting infection.

CONCLUSION

According to the methodology and the results found in this study, it was concluded that luminol solution is fully capable of detecting blood when compared to the naked-eye examination, thus being an important ally in the detection of traces of blood in dental instruments. The use of luminol has also validated the cleaning/sterilization process, since no traces of blood tissue was detected by this substance. Further studies with new formulations of luminol in alkaline solutions containing fluorescent additives are being developed to increase the limit of detection and selectivity for hemoglobin in dental equipment.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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