Research Article

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Comparison and Evaluation the Efficacy of Diode, Co_2 Lasers and Different Materials as Sterilization Methods on Contaminated Endodontic Files

El-Sayed AbdAllah El-Tayeb[1]*, Haytham S Moharrum[1], Doaa Mohamed Sadony[2] and Mohamed I Ebrahim[3]

[1]*Lecturer in the department of medical application of laser, National Institute of laser Enhanced science, Cairo University, Egypt*

[2]*Researcher in Restorative and Dental Materials Department, National Research Centre, El-Bohouth Street, Egypt*

[3]*Assistant professor of Dental Biomaterials, Faculty of Dental Medicine Al Azher University (Boys), Egypt*

ABSTRACT

Background: The reuse of endodontic files may lead to cross infection when these instruments become contaminated with micro-organisms, direct contact with blood, saliva and infected pulp tissue. Micro-organisms are considered a major cause in the failures of endodontic treatments. Proper sterilization of endodontic files is very important to prevent cross-infection from a patient to another one. This study was compared the effectiveness of different sterilization methods on contaminated endodontic files.

Material and Methods: The study was performed on 75 pre-sterilized stainless-steel K-files (25 mm) of size 25. The files were contaminated with a suspension of *Enterococcus faecalis* and were divided into five groups (n=15/each) based on the sterilization method. Group A: Autoclave, Group B: Glutaraldehyde, Group C: CO_2 laser and Group D: Diode laser, Group E: Control (no sterilization). After sterilization, the files were cultured on the agar plates to determine the bacterial account of total microbial reduction.

Results: The study showed that the files sterilized by autoclave, CO_2 laser, diode laser were completely sterile. The files sterilized by 2.4% glutaraldehyde were 77% sterile.

Conclusion: This study concluded that autoclave, diode and CO_2 laser are effective methods of sterilization in clinical practice.

KEYWORDS: Autoclave; Diode laser; Co_2 laser; Glutaraldehyde; Sterilization; *E. faecalis*

INTRODUCTION

The oral cavity is an environment rich with a variety of microorganisms. More than 700 bacterial sorts have been identified in the human oral cavity. Many of these bacteria may share in the development of caries, as well as gingival, periodontal and endodontic diseases [1-3].

In dentistry, especially *Enterococcus faecalis*, has associated with chronic periodontitis and failed root canal treatments involving chronic apical periodontitis. The root canals environment has a good condition for the survival of enterococci and the establishment of long-standing local infections, as the quality of obturation, could also influence the colonization of *E. faecalis* and

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Address for correspondence: El-Sayed AbdAllah El-Tayeb, Lecturer in the department of medical application of laser, National Institute of laser Enhanced science, Cairo University, Egypt

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microflora in roots, either directly or indirectly. Several studies tested the susceptibility of *E. faecalis* to endodontic treatment, which showed high resistance to antimicrobial agents. Also, it can survive in very harsh environments, with poor nutrient supply and high alkaline pH.

The reuse of instruments in the dental field carries a great risk of cross-contamination and cross- infection between patients and the staff at a dental clinic [4]. The instruments used in the dental field are in contact with saliva and blood which may contribute to transmission of serious infections like Hepatitis B, Hepatitis C and HIV. These infections can cause many complications for patients and dental staff [5-7]. It is the duty of medical professionals to ensure equipment safety and provide a clean environment to prevent transmission of microorganisms through improperly sterilized instruments [4].

Endodontic instruments are particularly often reused. Instruments like reamers, files, gates glidden drill and peeso reamers are used for removing infected pulp tissue, cleaning and shaping the root canal system, so that the bacterial population in root canal is removed. Residues on these instruments may acts as infectious agents and contribute to cross-infection from one patient to another [8-11].

Sterilization was preformed to remove, kill or deactivate all forms of microorganisms such as fungi, bacteria, viruses, and spores from an instrument [4,8], by using a physical or chemical procedure to destroy all microbial life, including highly resistant bacteria spores [4,12].

Chemical Sterilization used for microorganisms removal by using chemical bactericidal agents as Glutaraldehyde, Formaldehyde, and Phenols. In physical sterilization heat is the method of sterilization of objects. Physical methods are dry heat, moist heat, radiation and ultrasonic vibration [12]. The autoclave is the most used technique. For sterilization of endodontic instruments [4], using of lasers as carbon dioxide laser, diode laser, argon laser and Nd: YAG laser has been proposed in several researches and have been tested for this purpose [10,11,13,14]. The sterilization of endodontic files using carbon dioxide laser (CO_2) has been proven to be effective [10,11,14], which falls into the infrared range on the spectrum and has a wavelength of 10.600 nm.

CO_2 laser is greatly absorbed by tissues has highly water content. When CO_2 laser is used in focused mode, its energy is dense, and has a fine dissection. As the beam is defocused, widened, and its impacts on the tissue change. The laser ablates the tissue by superficial vaporization of cells and coagulates blood vessels that are smaller than the beam diameter. Also, diode laser for sterilization, the diode laser comes in different wavelengths (810,940 and 980 nm) are the most common, that allows for greater absorption by water than hard dental tissues. The energy is delivered by a fiber in contact mode. The main objective of this study was to investigate the effectiveness of various sterilization techniques applied to the used dental instruments including endodontic files because *Enterococcus faecalis* strain was played a role in resistant endodontic infections [15,16], in-which the following sterilization methods will be used: 1-Autoclave Sterilization, 2-Diode laser sterilization. 3-CO_2 Laser Sterilization, and 4-Chemical Sterilization with Glutaraldehyde.

MATERIALS AND METHODS

75 K files of size 25- and 25-mm length were used for this study. All the files in the study were pre- sterilized in endodontic instrument box by autoclave for 30 minutes/121 degree at 15-pound pressure to eliminate any bias. The test microorganism used in this study was a commercially obtained strain of *Enterococcus faecalis* (ATCC 29212). The *E. faecalis* strain was inoculated in nutrient broth and placed in an incubator to allow the bacteria to grow at 37 °C / 48 h. The 75 test files were divided into 5 groups 15 files for each group and labeled as follow:

Group A: 15 Files to be sterilized using Autoclave.

Group B: 15 Files to be sterilized using 2.4 % Glutaraldehyde solution

Group C: 15 Files to be sterilized using Carbon dioxide Laser (10.600nm)

Group D: 15 Files to be sterilized using Diode laser (980nm).

Group E: 15 Files used as a control; not subjected to any form of sterilization

For contamination, the pre-sterilized files were placed in the test tubes containing bacterial broths and incubated for 24 h / 37 °C.

Group A: files were placed in a sterilizing bag and subjected to autoclave sterilization at 121 °C /15 min at a pressure of 15 pounds.

Group B: files were placed in a sterile glass container containing 2.4% glutaraldehyde solution at pH=8.3 as per manufacturer's guidelines. (Cidex®, ASP) and left for 12 hours.

Group C: files were irradiated for 3 seconds/surface at 10 watts using carbon dioxide laser. During (3-second) time the laser beam was moved along the file length and rotating the file to irradiate all around. The files were held by using a tweezer to change the surface for exposure, while keeping the laser beam at 3cm fixed distance away from the samples.

Group D: files were irradiated for 3 seconds/surface at 10 watts using diode laser system. During (3-second) time the laser beam was moved along the file length and rotating the file to irradiate all around. The files were held by using a tweezer to change the surface for exposure, while keeping the laser beam at 3cm fixed distance away from the samples.

Group E: files were not subjected to any sterilization and were stored in an endodontic instrument box at room temperature. Following sterilization, the files were rinsed using 3 ml of distilled water and 100µl of the solution was transferred onto tryptic soy agar (TSB, Sigma-Aldrich, St. Louis, MO, USA) prepared petri plates and incubated at 37 °C/24 h. The same was performed for the control group. After the incubation period the colony forming units (CFU) for each group was counted using an automated bacterial colony counter.

RESULTS

The study showed that the endodontic files sterilized by autoclave sterilization (Group A) showed complete sterilization (98%), The files sterilized by immersion in 2.4% glutaraldehyde solution (Group B) showed a sterility of (77.42%), The files subjected to CO2 laser sterilization (Group C) and diode laser sterilization (Group D) for 3 seconds / surface at 10 watts also showed sterilization (97.2%), while control group (Group E), showed highly growth of bacteria. A comparison of the pre-sterilization and post-sterilization CFU values (CFU/ 100µl sample/ plate) for the five groups is shown in Table 1 and Figure 1.

Table 1: Pre-sterilization and post-sterilization CFU/ml counts.

Groups	*Enterococcus faecalis*				
	Pre- Sterilization CFU/ml Count (Mean ± SD)	**Post- Sterilization CFU/ml Count (Mean ± SD)**	**Reduction %**	***P*-value**	**S**
A (autoclave)	3 x 104 ± 0.37 x 104	0.06 x 104 ± 0.045 x 104	98	0.001	Highly significant
B (Glutaraldehyde)	3 .1 x 104 ± 0.41 x 104	0.7 x 104 ± 0.24 x 104	77.42	0.001	Highly significant
C (CO_2 Laser)	3.1 x 104 ± 0.41 x 104	0.08 x 104 ± 0.047 x 104	97.42	0.001	Highly significant
D (Diode Laser)	3 x 104 ± 0.27 x 104	0.08 x 104 ± 0.021 x 104	97.2	0.001	Highly significant
E (Control)	3 x 104 ± 0.37 x 104	3.1 x 104 ± 0.42 x 104	-3.33	0.21	Not significant

SD: standard deviation %: percentage *P*- value: probability S: significant

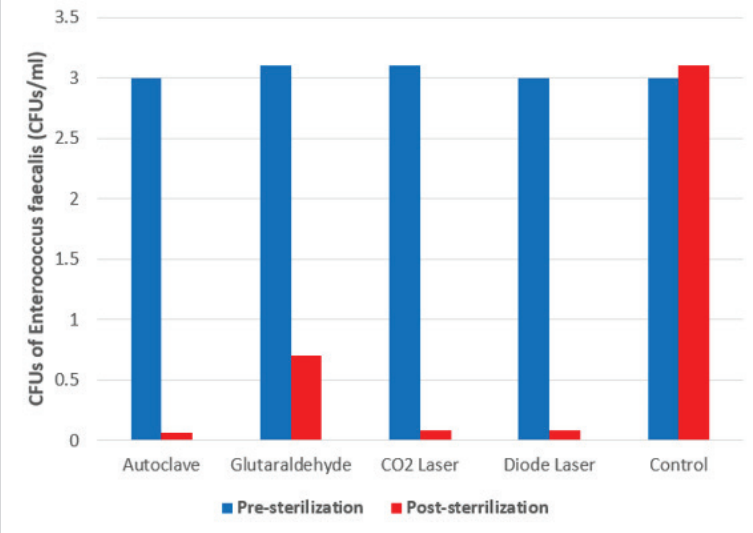

Figure 1: Bar chart mean values of colony forming units (FCUs/ml) of *Enterococcus faecalis* pre and post sterilization in the 5 groups.

DISCUSSION

The study was done to evaluate effective and fast method to sterilize endodontic file in clinical set up. Endodontic files undoubtedly retain bacteria and biological debris from the root canal due to their intricate design and complex architecture. So, proper sterilization is importance to prevent cross-infection, ensure patient safety and success of the root canal treatment.

Root canal infections have a polymicrobial nature. *Prevotella* species, *Fusobacterium, Spirochetes* and gram-positive bacteria as *E. faecalis* and many other microorganisms have been identified in endodontic infections [16-19]. E. faecalis was playing an important role in endodontic infections. It has been associated with primary as well as secondary (failed/unsuccessful treatment) endodontic infections [15,18,19]. *E. faecalis* can survive in environments reaching a pH up to 11.5 with poor nutrient supply and has even been reported to survive in 6.5% concentrated sodium hypochlorite solution [15]. Due to these characteristics, it was chosen as the test microorganism for this study. Autoclaving is the main method for sterilization method in the dental practice. The heat that delivers via pressurized steam kills bacteria and other microorganisms by causing organisms' structural proteins and enzymes lose their shape in an irreversible way, denaturing, coagulating and making them nonfunctional. It is efficient for the destruction of bacteria, viruses and spores. Autoclaving relies on exposing the instrument directly to steam at a specific pressure, temperature and for a specific time [4,12,20,21]. Complete sterilization was achieved in the autoclave group (Group A) in this study. Sterilization of endodontic files at 121 °C /15 min at a pressure of 15 pounds has been effective and the results of this study are in agreement with the existing literature [8,11,20,22].

The endodontic files sterilized by immersion in 2.4% glutaraldehyde for 12 hours (Group B) showed 77% sterilization. Glutaraldehyde sterilization relies on its broad-spectrum activity due to its ability to destroy bacteria, viruses, fungi and spores [23]. Glutaraldehyde acts by proteins denaturation and alkylation of nucleic acids of bacteria [9]. The efficacy of glutaraldehyde sterilization is however controversial in literature [8]. There are several studies that report incomplete sterilization using 2.4% glutaraldehyde, with sterilization rates ranging between 60-80% [9,11,22] as the result of our study. It pointed that the efficacy relies on the time of exposure of the instrument to glutaraldehyde solution and prolonging the immersion period may lead to complete sterilization. In the present study, CO_2 laser (Group C) achieved sterilization of endodontic files. *E. fecalis* was preferred in this study in order to investigate the effect of the laser heat, because of its resistant to heat. These findings are in agreement with the results of the studies performed by Raju et al. [11] and Venkatasubramanian et al. [10]. In a study performed by Al-Jamell et al. [14] however, endodontic files sterilized by CO_2 laser were only 93.85 % sterile. Sterilization by CO_2 laser is based on the absorption of the laser beam by cells/micro-organisms resulting in their vaporization [24].

Sterilization of endodontic files using CO_2 laser may be seen as a less time-consuming and chair side alternative to autoclaving. It must however be noted that the CO_2 laser cannot be used on materials that are particularly thermo-sensitive, like the color-coded handle of the endodontic file. In the present study contamination of the working blade only was performed in a laboratory setting. Laser sterilization was only done on the contaminated working blade made of stainless steel. The plastic handle of the file was not exposed to the laser beam to avoid melting and damage of the instrument. Care was taken to manipulate the handle only with sterile tweezers throughout the laser sterilization process to prevent false results. In study performed by Al-Jamell et al. [14], the plastic instrument handle was even removed from the instrument shaft to overcome this problem. The same for the diode laser which can sterilize at the lowest energy level (97%) due to its thermal and photo-disruptive effects resulting in sublethal damage (disruption of cell wall integrity and accumulation of denatured proteins) and subsequent cell lysis and death [25-29]. Diode laser was used in the present experiment as it is one of the more commonly used modes of laser in the dental office [30,31].

CONCLUSION

Proper sterilization of endodontic files is necessary to prevent cross- infection between the patients. In this study the autoclave and laser sterilization showed complete sterilization. Glutaraldehyde solution achieved a 77 % sterilization of the endodontic files. Autoclave sterilization remains an effective, reliable and verifiable method. In a dental practice, with a CO_2 laser device, diode laser sterilization is a time-saving and effective alternative.

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