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Research article

Impact of using Single File System Versus Multiple File System on species bacterial biofilm in Oval Canals

(Comparative in- vitro Single-study)

Abeer Hashem Sayed Mahran[1], Soha Abdel rahman Elhady[2], Mohamed Turky Abdel Razik[3] and Ebthal Hamada Saleh[3]

[1] Department of Endodontics, Faculty of Dentistry, Ain Shams University
[2] Department of Microbiology, Faculty of Medicine, Ain Shams University
[3] Department of Endodontics, Faculty of Dentistry, Minia University

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Abstract:

Aim of Study: The current study aimed to assess and evaluate the effects of a single file system against a multiple file system on the eradication of single-species E. faecalis biofilm in oval canals. Additionally, the study examined the influence of various additional approaches on the efficacy of these file systems. **Materials and Methods:** A total of 100 human Maxillary second premolars were acquired from the outpatient clinic. All collected teeth (n=100) were numbered and randomly assigned to four groups as follows: In Group I, consisting of 40 samples, instrumentation was performed utilizing a single-file method known as Reciproc Blue 40, accompanied by saline irrigation. Group II (n=40): The samples were instrumented using the Hyflex CM multiple file system, with Saline irrigation. Group III (n=10): A positive control group was employed to assess the survivability of bacteria during the entire experiment. In this group, the root canals were intentionally contaminated but not instrumented. Group IV (n=10): A negative control group was included in the study to assess the sterility of the procedures. In this group, the root canals were instrumented but not contaminated. To introduce E. faecalis suspension into each root canal, 20 lL was filled through their orifice using sterile automatic micropipettes. The bacterial colonies were enumerated, and the resulting data was quantified as colony-forming units per milliliter (C.F.U./mL). The results indicate a statistically significant difference seen among the various groups ($p<0.001$). The positive control exhibited the highest value (12.39±0.16), followed by Reciproc blue (11.90±0.21) and Hyflex CM (10.95±0.36), whereas the negative control had the lowest value (0.00±0.00). All post hoc pairwise comparisons yielded statistically significant results ($p<0.001$). **Conclusion:** The study's findings indicate that the implementation of a multi-file system resulted in a greater reduction of bacteria compared to a single-file system.

Key words: B , M , S

Introduction

Microorganisms, particularly bacteria, significantly contribute to the pathogenesis of pulp and periapical disorders, and they also exert influence on the prognosis of endodontic therapy1. Enterococcus faecalis, also known as E. faecalis, is a type of bacteria that exhibits facultative gram-positive characteristics. This bacterium possesses a high level of resistance to antimicrobial agents, enabling it to effectively withstand their effects. Additionally, E. faecalis has the ability to stick to, develop inside, and penetrate dentinal tubules. Furthermore, it demonstrates resilience against the host's defense

mechanisms. Consequently, this particular coccus has the potential to be the causative agent behind enduring endodontic lesions. The user's text is too short to be rewritten academically.

The utilization of mechanical equipment in root canals is a significant factor in reducing intracanal bacterial presence (3,4).In the field of endodontics, it has been a regular practice for practitioners to utilize manual instrumentation for the purpose of sculpting root canals during treatments. The evolution of nickel-titanium (NiTi) engine-driven files offers several advantages compared to manual instrumentation, including increased procedural speed, improved centering of preparations, and reduced apical extrusion of debris.

The introduction of thermomechanically treated engine-driven files has led to significant advancements in root canal instrumentations. Hyflex CM and Reciproc Blue are illustrative instances of thermomechanically processed files, fabricated from CM wire. The Controlled Memory Effect facilitates the file's ability to maintain its original shape even when it is removed from the canal. This particular characteristic is accountable for the prevention of procedural problems, including but not limited to the production of ledges, transportation issues, and perforations. The exceptional flexibility exhibited by these files renders them highly desirable in the context of curved canals.

Materials and Methods

Selection of the samples:

One hundred Maxillary second premolars were obtained from the outpatient clinic at Minya University's Faculty of Dentistry. These premolars had been recently extracted due to periodontal concerns. The teeth underwent a cleaning process to remove both soft and hard tissue attachments, followed by immersion in a sodium hypochlorite solution for a duration of 30 minutes. The teeth were then cleansed and submerged in a 0.1% thymol solution until they were ready for utilization. To ensure impartiality, a meticulous approach was employed in selecting teeth with root canal structure that closely resembled each other in both the mesiodistal and buccolingual dimensions. This involved capturing periapical radiographs in a direct manner.

Samples Classification:

All collected teeth (n=100) was numbered, randomly assigned into the following four groups:

Group I consisted of 40 samples that were instrumented utilizing a single-file system, specifically Reciproc Blue 40, with the use of saline irrigation.

Group II (n=40): The samples were subjected to instrumentation utilizing the Hyflex CM multiple file system, with saline irrigation.

Group III (n=10): A positive control group was employed to assess the survivability of bacteria during the course of the experiment. In this group, the root canals were intentionally contaminated but not instrumented.

In Group IV, consisting of 10 subjects, a negative control group was employed to assess the sterility of the operations. In this control group, the root canals were instrumented but deliberately kept free from any contamination.

Prior to instrumentation, the Hyflex CM and Reciproc Blue instruments were subjected to autoclaving at a temperature of 121 °C for a duration of 20 minutes.

The instruments in the Single File System group (Reciproc Blue) were utilized in a crown down procedure with a reciprocation frequency of 10 cycles per second, which is approximately comparable to 300 revolutions per minute (rpm). The Reciproc instrument was employed in the root canal using a deliberate in-and-out pecking motion, while ensuring that the instrument was not fully withdrawn from the canal. Additionally, it was utilized in a brushing motion along the lateral walls of the root canal. Initially, the instrument was employed until it had attained two-thirds of the predetermined working length. Subsequently, the instrument was extracted from the canal, followed by irrigation of the canal. The instrument was then reused in a

similar fashion along the working length. Following the completion of the instrumentation process, the canals were subjected to another round of irrigation. The instrumentation of each root canal was performed using a Reciproc blue file RB40, which has a size of 40. Each individual tool was employed for the specific purpose of preparing a single canal. The adjustment of torque and speed will be carried out in accordance with the instructions provided by the manufacturer.

The suggested instruments for the Multi-File System group (Hyflex CM) consist of three instruments, as specified by the manufacturer. The process of coronal shaping involves the utilization of several file sizes. Initially, a file with a size of 0.08/25 will be employed, followed by a 0.04/25 file till reaching the working length (WL). Subsequently, a 0.06/20 file will be utilized for shaping the middle-third. Finally, files with sizes of 0.04/30 and 0.04/40 will be employed to shape the entire length. The files will be utilized at a rotational speed of 500 revolutions per minute (rpm), with a torque of 2.5 Newton centimeters (Ncm), employing a handpiece with a 16:1 reduction ratio. This will be done in a sequential pecking motion, without applying any pressure at the apex.

Samples preparation:

A sterile round diamond bur was utilized to prepare the conventional access cavity, which was then refined utilizing a sterile safe-ended tapered carbide fissure bur attached to a high-speed handpiece. This process aimed to establish a straight-line access to the canal orifices, adhering to the established guidelines for access cavity preparation.

Following the completion of access cavity preparation, the pulp chamber underwent irrigation and immersion in a solution of 5.25% sodium hypochlorite (NaOCL), after which the working length was determined.

The sealing of the apex was accomplished using composite resin, while nail polish was put to the root surface as a measure to prevent bacterial leakage. Cotton pellets shall be carefully positioned onto the canal orifice and subsequently secured by means of a temporary filling.

In order to enhance the ease of handling and identification, the teeth were vertically embedded in acrylic resin and subsequently positioned into Eppendorf tubes.

After the acrylic glue had set, the temporary filling and pellet were subsequently removed. The tubes were carefully positioned within plastic carrier boxes, which were thereafter enclosed with autoclave sachets. The entire assembly underwent autoclaving for a duration of 15 minutes at a temperature of 121 °C. The effectiveness of sterilization was assessed by employing an indication that was inserted into the sachets.

Prior to contamination, the specimens were placed within 20 mL Falcon tubes filled with distilled water and subjected to sterilization in an autoclave at a temperature of 121°C for a duration of 15 minutes.

In order to assess the sterilization of the specimens, a sterile saline solution was used to irrigate each specimen, with 1ml being used. Subsequently, a culture was obtained by inserting three sterile size 15 paper points into the root canals in a sequential manner, with each point being left in place for a duration of 1 minute.

The samples were inoculated onto Mitis Salivarius agar plates and incubated at a temperature of 37 degrees Celsius for a duration of 48 hours.

The specimens were contaminated with E. faecalis (ATCC 29212), which had been previously cultivated in brain-heart infusion broth obtained from HiMedia Lab. Pvt. Ltd. in Mumbai, India. The culture of E. faecalis was then grown on 5% blood agar plates also obtained from HiMedia Lab. Pvt. Ltd.

The lyophilized strain of E. faecalis (ATCC 29212) was subjected to a washing

procedure using 2 mL of phosphate-buffered saline (PBS) with a pH of 7.2. Subsequently, a microbial suspension of 0.1 mL was cultivated in 2 mL of brain-heart infusion broth (BHIB) from HiMedia Lab., Pvt., Ltd. The resulting mixture was then inoculated onto a blood agar base supplemented with 5% sheep blood, also from HiMedia Lab., Pvt., Ltd.

Subsequently, the samples were subjected to incubation for a duration of 24 hours at a temperature of 37 degrees Celsius. The confirmation of microbial strains was achieved by the utilization of Gram's stain as well as the observation of colonial and growth properties.

The colonies of E. faecalis were collected from the medium and resuspended in a 4 mL solution of phosphate-buffered saline (PBS).

The microorganisms were diluted to achieve a suspension with an estimated concentration of 108 colony-forming units per milliliter in sterile phosphate-buffered saline (PBS) by employing McFarland standard tubes number 1.

The quantification of viable microorganisms per milliliter was accomplished by measuring the optical densities of cultures at a wavelength of 600 nanometers per liter (OD600) using a spectrophotometer manufactured by IMPLEN in Munich, Germany.

The microbial suspensions were subjected to vortexing for a duration of 1 minute in phosphate-buffered saline (PBS). Following a series of 10-fold dilutions in phosphate-buffered saline (PBS), 0.1 mL samples were dispensed onto blood agar plates and incubated at a temperature of 37 °C for a duration of 24 hours.

The experiment involved the cultivation and enumeration of colony-forming units, followed by the computation of a logarithmic transformation.

A volume of 20 microliters (lL) of E. faecalis suspension was introduced into each root canal through their orifice using sterile automated micropipettes.
The bacterial suspension was transported to the working length using sterile K size 15 files.

Subsequently, the tubes containing the roots were transferred into sterile plastic carrier boxes and subjected to incubation at a temperature of 37 °C for a duration of 24 hours.

Following the contamination of the root canal with E. faecalis ATCC, the teeth were subjected to random assignment into four distinct groups. These groups consisted of two experimental groups, one positive control group, and one negative control group. The experimental groups were subsequently separated into four subgroups based on the instrumentation technology and irrigation protocols employed.
Bacterial sampling following instrumentation.

Following the completion of root canal instrumentation, it is customary to introduce three sterile paper points, each with a size of 15, into each individual root canal. These paper points are left in place for a duration of one minute per canal. The specimen was carefully transferred into tubes using aseptic tweezers, and each tube was filled with 1 mL of physiological saline solution. The contents of the tubes were then mixed vigorously for a duration of 30 seconds using a vortex mixer. Following a 10-fold serial dilution in saline, 20 microliter aliquots will be distributed onto Mitis Salivarius agar plates and subsequently incubated for a duration of 24 hours at a temperature of 37 degrees Celsius. The bacterial colonies were enumerated, and the outcomes were quantified as colony-forming units per milliliter (C.F.U./mL).

Results

1- Effect of filing system:

Saline:

There was a significant difference between different groups ($p<0.001$). The highest value was found in positive control (12.39±0.16), followed by Reciproc blue

(11.90±0.21), then Hyflex CM (10.95±0.36), while the lowest value was found in negative control (0.00±0.00). Post hoc pairwise comparisons were all statistically significant ($p<0.001$).

Discussion

Enterococcus faecalis is a type of coccus bacterium that is classified as gram-positive. It is characterized by its nonfastidious nature, meaning it does not require specific or complex growth conditions and is able to thrive under a wide range of environmental conditions. The bacteria under consideration is a facultative anaerobe, possessing the capability to infiltrate dentinal tubules. This intrusion has the potential to result in a substantial infection. Moreover, Enterococcus faecalis exhibits the capacity to adhere to collagen in the presence of human serum, so enabling the preservation of its survival mechanisms.

The choice of this specific microorganism as the bacteriological indicator in this study was made due to its well-documented resistance to chemomechanical techniques. In this particular scenario, the presence of persistent endodontic infections and periradicular inflammation is commonly reported. The species under consideration demonstrates a notable degree of resilience within the oral cavity, showcasing the ability to persist even under unconventional environmental circumstances marked by restricted food resources. Moreover, the relative ease with which it may be cultivated and manipulated enhances its suitability for research purposes.

The primary objective of the present study was to examine and analyze the impact of a solitary file system in comparison to a multiple file system on the complete elimination of E. faecalis biofilm composed of a single species in oval canals. Furthermore, the investigation explored the impact of diverse supplementary methodologies on this phenomenon.

To ensure uniformity among the specimens, maxillary second premolar teeth were chosen as they possess root canal structures that may influence the outcomes of microbiologic load analysis.

The classification of root canal configurations in cross-sectional perspectives includes various shapes such as round, oval, extended oval, flattened, and irregular channel shapes. Oval canals demonstrate a buccolingual diameter that is twice the size of their mesiodistal diameter, whereas elongated oval canals exhibit a buccolingual diameter ranging from two to four times more than their mesiodistal diameter.The numerical value of 15.
The selection of oval-shaped canals in this study was driven by their capacity to pose a significant challenge to medical professionals.

The optimal preparation generally demonstrates a circular cross-sectional shape and generates uninstrumented cavities in the forms of ovals, elongated ovals, and flattened canals. The recesses have the potential to harbor robust bacteria, which may undermine the efficacy of the treatment. Bacteriological sampling was performed using sterile paper points. The utilization of paper points for sampling purposes is associated with a number of restrictions. This particular technique alone permits the collection of microorganisms that are found in the root canal, while those that inhabit the dentinal tubules remain unidentifiable.

Before initiating the sample procedure, a manual tool was utilized to perform a pumping motion along the root canal. This technique aimed to enhance the reliability and predictability of the sampling process, particularly in narrow canal recesses.The numerical value of ten.

The access cavity in the maxillary premolar was intentionally left intact to serve as a reservoir for irrigant solution, in accordance with the experimental circumstances outlined in the experiments done by Alves et al. (2014) and Carvalho et al.The user has entered a numerical value of 15.

The primary methodology employed for assessing the existence of bacteria involves quantifying colony-forming units (CFUs) by culture techniques, which is later supplemented by molecular methodologies such as DNA detection. The application of molecular methods has resulted in increased sensitivity. The selection of the CFU counting method for our analysis was based on its effectiveness and simplicity, which is consistent with the methodology employed in the Andac18 study. The Hyflex CM is a multifile system that demonstrates enhanced flexibility as compared to alternative instruments comprised of super-elastic wires. Instruments exhibiting a high degree of flexibility experience a decrease in their ability to effectively cut inside the root canal walls, as they are prone to distortion even under moderate pressure. This susceptibility to deformation has the potential to negatively affect the reduction of bacteria within the root canal.The numerical value provided by the user is 16. The Reciproc Blue is a unique file system that utilizes reciprocating motion, and it was developed by VDW in Munich, Germany. The product integrates a recently developed alloy, fabricated via a thermomechanical manufacturing technique, resulting in the formation of a unique oxide surface layer.

The findings of this study were in line with the results given by Carvalho et al. (2015), but were in disagreement with the conclusions drawn by Basmaci et al. (2010) due to the use of mandibular premolar teeth with a single root canal.

Conclusion

The results of this investigation suggest that Hyflex CM has superior efficacy in reducing bacterial presence in oval canals compared to saline irrigation.

Recommendation :

It has been suggested that incorporating supplemental techniques such as H-files and ultrasonic irrigation (using a 5.25% sodium hypochlorite solution) may be beneficial.

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