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The Effectiveness of Three Instrumentation Techniques on the Elimination of Entererococcus Faecalis from a Root Canal: An In Vitro Study

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Abstract

The *in vitro* reduction of a bacterial population in a root canal by mechanical instrumentation using three techniques was evaluated. Root canals inoculated with a *Enterococcus faecalis (E. faecalis)* suspension were instrumented using hand Hedstroem files, Giromatic files, and Hero 642 rotary instruments. Irrigation was performed using sterile saline solution. Root canals were sampled before and after instrumentation. After serial dilutions, samples were plated onto *Mitis-Salivarius* agar and the colony forming units grown were counted. All instruments tested were able to significantly reduce the number of bacterial cells in the root canal, however, the results of this study indicated that Hedstroem files, Giromatic, and Hero 642 techniques were not significantly different in their ability to reduce intracanal bacteria.

Keywords: Hero 642, Giromatic, Hedstroem files, root canal microbiology, root canal instruments

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Introduction

The cleaning of dentin within the root canal and the removal of inflamed and/or necrotic tissue remains as one of the most important steps in endodontic therapy.[1] It has been demonstrated eradication of endodontic infection enhances the success rate of the endodontic therapy.[2]

In infected root canals reducing the bacterial count is accomplished by a combination of mechanical instrumentation, various irrigation solutions, and antimicrobial medicaments or dressings placed into the canal. Chemomechanical instrumentation is often the first means of bacterial reduction during endodontic treatment of infected root canals.[3, 4] Previous studies in which no antibacterial irrigants were used reported the mechanical action of instrumentation and irrigation was effective in reducing the number of bacterial cells in the root canal.[3, 4]

Classic bacteriological studies were performed using stainless-steel hand instruments with a filling or reaming motion.[5] The disinfection efficacy should, therefore, become one of the considerations in development of new instrumentation techniques to be used in root canal therapy. For example, studies by Kerekes and Tronstad describing the morphology of apical root canal anatomy and the standardized instrumentation technique concluded that to achieve adequate debridement and root end preparation use of larger files apically than those commonly used in practice today was necessary.[6-9]

Soon after the introduction of nickel-titanium (NiTi) endodontic hand instruments in 1988, NiTi rotary instrumentation became popular.[10] In modern endodontic practice there was a move towards the use of engine-driven rotary instrumentation with NiTi files. Many clinicians argue this form of instrumentation allows for easier production of standardized root canal preparations, thereby, yielding superior debridement, in part because of NiTi instrument flexibility.

Siqueira and coworkers[11] found no difference in bacterial reduction when comparing root canal instrumentation with profile #5 instruments and either GT files (Dentsply/Tulsa Dental, Tulsa, OK, USA) or hand K-files to a size #30. However, this study did in fact show that enlargement to a size #40 stainless steel K-file was significantly more effective at reducing bacterial levels than the two other rotary instrumentation techniques. These two studies used microbiologic culturing techniques to detect the remaining bacteria from infected root canals with different instrumentation techniques.[4, 11]

The super elastic property of NiTi, coupled with advanced file design, allowed safe and effective instrumentation using hand piece-driven files operated at slow speeds in a crown to apex direction. NiTi instrumentation has been proven effective for maintaining the original canal shape.[12]

NiTi instruments may be as aggressive or better than stainless-steel files in removing dentin, and they are more resistant to wear than their stainless-steel counterparts.[13]

The removal of bacteria by NiTi instrumentation was evaluated in two recent studies by Dalton et al.[4] and Shuping et al.[14] The results from Dalton et al.[4] mirrored those of Byström and Sundqvist.[15] They found significant bacterial reduction with samples free of bacteria. Also, no significant difference was found between the instrumentation with either stainless steel hand files or rotary NiTi instrumentation.

Recently, NiTi files with increased tapers and different designs have been developed. The Hero 642 (Micro-Mega, France) concept consists of a series of instruments of three apical diameters corresponding to ISO norms 20, 25, and 30/100th, combined, for each of these three diameters, with three tapers .06, 04, and 02.[16]

Investigators have evaluated the efficacy of different preparation techniques in cleaning the apical region of the root canal.[17, 18] Wu and Wesselink[17] compared the efficiency of three techniques using K-files in cleaning the apical portion of curved root canals.

Siqueira et al.[18] compared the effectiveness of five instrumentation techniques for cleaning the apical third of root canals using histological evaluation method. Canals were prepared with the step-back technique using stainless steel or Ni-Ti files, an ultrasonic technique using a ISO size 15 ultrasonicfile, balanced force using Flex-R-files, and Canal master U instruments.

The purpose of this study was to compare the intracanal bacterial reduction provided by instrumentation using Hand file (Hedstroem file), Giromatic, and Hero 642.

Materials and Methods

Thirty-five extracted human lower bicuspids with a single root canal were used for this experiment. Bone, calculus, or soft tissues on the root surface were removed with curettes. Teeth were stored in tap water prior to experimental procedures.

Conventional access preparations were made and the root canals were instrumented 1 mm beyond the apical foramen with K-type files up to size 30 and then flared.

Irrigation with tap water was performed during the enlarging procedure. After root canal preparation, the enlarged apical foramen was sealed by means of epoxy resin to prevent bacterial leakage. To make both handling and identification easier, the teeth were then mounted vertically in plaster blocks and sterilized overnight by ethylene oxide gas.

A suspension was prepared by adding 1 ml of a pure culture of *Enterococcus faecalis (E. faecalis)* (ATCC2912), grown in brain heart infusion broth. Each root canal was completely filled with the *E. faecalis* suspension using sterile pipette. Sterile K-type files #15 were used to carry the bacterial suspension to the working length (WL). The blocks were incubated at 37°C for 24 h.

After incubation, the root canals were divided into three groups accordingly to the instrumental technique used as follows:

- **GROUP 1:** Five root canals were used as a control group. In this control group the pulps were extirpated but no instrumentation or irrigation was done.
- **GROUP 2:** Ten root canals were handly-instrumented using Hedstroem files. A #15 file was placed to length and a combination of a filing and a reciprocal reaming action was used until it fit loosely in the canal. This was repeated with successively longer files until the apical portion of the canal was instrumented to a #40 file.
- **GROUP 3:** Ten root canals were enlarged using Giromatic. The giromatic hand piece works with a reciprocal quarter turning motion and was used with Hedstroem files. (ISO15-30).
- **GROUP 4:** Ten root canals were instrumented using Hero 642 rotary instruments in a crown-down manner. Because the angle of curvature was below 10° (based on Schneider's simple criteria)[19] the Hero blue wave was used with a .06 taper no 30, .04 taper no 30, and .02 taper no 30 in a crown-down technique. A .06 taper Hero no 30 was placed in the root canal and the black silicone was stopped at half or two thirds of the WL proceeded with short-and-fast, in-and-out movements. Then place a .06 taper Hero no 30 in the root canal and stop the gray silicone at WL minus 2 mm. Proceed with short-and-fast, up-and-down movements. Lastly, place a .02 taper Hero no 30 in the root canal. The white stop adjusted the WL. The preparation of the canal was performed in the same way as mentioned above.

Each root canal was irrigated with a total volume of 10 ml of 0.85% sterile saline solution. Irrigation was delivered in the canals by means of a 3 ml plastic syringe with a 23-gauge needle.

Paper points used to sample the canals were transferred into tubes containing 1 ml of 0.85%

Table 1. Mean values of the quantity of bacterial cells in the root canal before and after instrumentation using the different files.

	Mean	Standard Deviation	N
K-File	10	3.0535	10
Giromatic	8	2.7386	10
Hero 642	8	2.7386	10

saline solution and vortexed for 1 min. After 10-fold serial dilutions in saline, aliquots of 0.1 ml were plated onto *mitis salivarius* agar plates and incubated at 37°C for 48 h. The colony forming units grown were counted and a log transformation calculated.

Data obtained from samples taken before and after instrumentation were analyzed statistically for differences inside groups using the paired t-test and between groups by means of the nonpaired *Kruskal-Wallis* test. The significance level was established at 5% ($p<0.05$).

Results

The mean number of bacterial colonies in the initial samples from the root canals prepared by the K-file averaged 80 colonies. After instrumentation with Hedstroem files, the mean values of the number of bacterial colonies decreased to 10 colonies.

Following instrumentation with Giromatic files, the mean values of the number of bacterial colonies decreased to 5 colonies. After instrumentation with Hero 642, the mean values of the number of bacterial colonies decreased to 5 colonies. Data are summarized in Table 1.

By comparing the samples taken before and after instrumentation, it was possible to observe all instruments tested were able to reduce significantly the number of bacterial cells in the root canal. All instruments tested were able to reduce significantly the number of bacterial colonies in the root canals.

Discussion

In the past decades several authors provided evidence that bacterial infections play a decisive role in the course of pulpitis and periapikal inflammation. Accordingly, for therapy, the reduction or eradication of the bacterial population seems to be a justified goal. On the basis of the majority of publications, it seems the optimal clinical result can be obtained when, immediately before canal filling, the root canal is germ-free or infected to a minimal degree and, thus, gives negative results with routine cultural techniques.

Step-back instrumentation using stainless-steel K-files was chosen as a comparative technique because it is frequently taught in dental schools and is commonly used by many practitioners. The relatively new NiTi rotary technique is gaining in popularity supported by the results of mechanical evaluations and user friendliness.

E. faecalis, a facultative anaerobic gram (-) coccus, has been recovered from oral sites.[20] *E. faecalis* was chosen as the bacteriological marker in this study. It is a non-fastidious, easy-to-grow aerobic bacterium of significant clinical importance that could be used in a study applying a bacteriological assessment method. Other bacteria commonly associated with endodontic infections may require symbiotic support from other bacteria, but *E. faecalis* has been reported to survive and successfully thrive alone, is heat resistant, and can survive at 60°C for 30 min.[21, 22]

Endodontic infections with *E. faecalis* usually cause a problem with treatment because this microorganism is difficult to elimitate. *Mitis salivarius* agar was used because it allows the growth of some *enterococci* and *streptococci*, including *E. faecalis*. The 0.85% saline solution has no antibacterial effects on *E. faecalis*. Because no antibacterial irrigant was used, elimiation of bacteria was just dependent on the mechanical action of instruments.

The endodontic literature contains numerous studies on automated root canal preparation, investigating working safety, preparation time, and straightening as well as root canal cleanliness.[23]

Recent technological advances have provided rotary instruments and instrumentation systems that can significantly improve the shaping of the root canal, particularly curved canals. Nevertheless, evidence suggests rotary instrumentation does not provide better results in either cleaning or disinfection capabilities when compared with hand techniques.[4, 24] More importantly, NiTi instruments can predictably enlarge curved root canals, while maintaining the original path, to sizes not routinely attainable with stainless steel files.

Byström and Sundgvist[4] reported samples taken after hand filling with saline irrigation usually contained 102 to 103 fewer bacterial cells than initial specimens. In some cases the reduction was even higher. Ørtstavik et al.[3] reported similar findings.

Dalton et al.[4] showed with larger instrument sizes the bacterial count decreased. This seems logical, and it would be interesting to see if even larger file sizes could get the bacterial count closer to 0. If so, it may be easier to reach these larger sizes with minimal procedural mishaps using NiTi rotary instrumentation. Other clinical effects of larger instrumentation including compromised restorability, fracture susceptibility, and canal path alternations must also be considered when using any instrumentation technique.

Bolanos et al.[25] compared sonic instrumentation with hand instrumentation and the Giromatic system. The sonic preparation resulted in the best clean-curved canals. Following hand instrumentation, debris was found in all parts of the canals. Hand instrumentation was slightly superior to Giromatic preparation.

The Endolift and the Giromatic handpiece have been shown to leave uninstrumented canal wall areas as well as massive debris and a smear layer in several investigations.[26-28]

Dalton et al.4 found no significant difference in intracanal bacterial reduction detected using either a .04 taper NiTi rotary or stainless-steel K-file hand instrumentation with sterile saline as the irrigant.

Esposito and Cunningham[29] noted hand and rotary NiTi instruments can predictably enlarge curved root canals, while maintaining the original path, to sizes not routinely attainable with stainless-steel files.

Siqueira et al.[30] found the mechanical effects cause a significant decrease in bacterial cell numbers in the root canal. The most bacterial reduction was obtained after larger preparation. Because mechanical means are insufficient to completely eradicate root canal infection, the use of adjunct substances processing antibacterial becomes necessary.

Conclusion

In the present study there were no significant differences between the Hero 642, Giromatic, and Hand files. The results of this study showed these mechanical effects cause a significant decrease in bacterial colony numbers in the root canal. Although all of the instrumentations yielded marked reductions in bacterial content, a total absence of bacteria could not be achieved by mechanical preparation.

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