The Seal Ability of Mineral Trioxide Aggregate and Nano Hydroxyapatite Retrograde Filling Material on Internal Apical Bacterial Leakage of Infected Root Canal (an in-Vitro Study)

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Abstract

**Background:** Most endodontic failures occur as a result of leakage of microorganism and their by-products from pathologically involved filled root canal system. Surgical endodontic therapy with retrograde filling is indicated to obtain an apical seal and save the tooth.

**Aim:** To evaluate the sealing ability of Mineral Trioxide Aggregate (MTA) and Nano Hydroxyapatite (NHA), when used as retrograde filling materials, and the effect of obturation on internal apical bacterial leakage of infected root canal.

**Material and Methods:** 100 recently extracted mature human single-rooted teeth were collected for this study. The crowns were removed and canals were prepared, and then sterilized by autoclaving. 10µl of the bacterial suspension was carefully inoculated into the instrumented canal of each specimen coronally, Apical 3mm were resected at 90 degree to the long axis, then 3mm deep retrograde cavity (class I) was prepared. The prepared samples were secured with wax within the hole of the cap of an eppendorf tube containing sterile manitol salt broth.

**Results:** No significance difference was observed between MTA and NHA.

**Conclusion:** NHA could be used as an alternative to MTA, being more cheap and available than MTA.

**Key Words:** Bioceramic – Bacterial apical leakage – Nanotechnology.

Introduction

**THE** main objective of all endodontic treatment is proper cleaning, shaping, and complete obturation of root canal system; thus prevent the proliferation of microorganism and that by-products and obtain a hermetic seal between the periodontium and root canal foramina, which considered the key for long term successful treatment.

Most endodontic failures occur as a result of leakage of microorganism and their by-products from pathologically involved filled root canal system and destroy periradicular tissue. When failures occur, surgical endodontic therapy with retrograde filling is indicated to obtain an apical seal and save the tooth. This study was evaluated and compared the sealing ability on the internal apical bacterial leakage of non-vital infected root canal using Nano Hydroxyapatite (NHA) and Mineral trioxide aggregate (Pro Root MTA) as retrograde filling materials.

**Material and Methods**

The teeth was collected from Ismailia General Hospital, Dental Clinic and the samples was prepared and examined in the Microbial Lab of Faculty of Medicine, Suez Canal University since 2012.

One hundred recently extracted mature human single-rooted teeth were collected for this study. The crowns were removed so that all roots segments were standardized to about 17mm Long. All canals were prepared to #40 master apical file, then sterilized. 10µl of the staphylococcus aureus bacterial suspension was carefully inoculated into the instrumented canal of each specimen coronally and incubated at 37°C for 48 hours. The prepared 100 specimens were classified to experimental (n=80), and controls (n=20). The experimental specimens were equally group as following; Group A: Non-obturated canals either with gutta-percha or end fill sealer, Group B: Obturated canals with gutta-percha and end fill sealer.

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The apical 3mm were resected at 90 degree to the long axis, then 3mm deep retrograde cavity (class I) was prepared.

Each Group (A,B) was subdivided into 2 equal subgroups according to tested retrograde filling materials Fig. (1).

Each specimen was secured with wax within the hole of the cap of an eppendorf tube containing sterile Manitol salt broth to a level sufficient to cover the sectional apical parts with its corresponding retrograde filling material, and placed in an incubator at 37ºC, for designated checkup at 3, 9, 30, 60, and 90 days.

At the end of each interval, the specimens which showed bright yellow color with turbidity Fig. (2) were recorded for each subgroup and bacterial count was performed using spectrophotometer Fig. (3).

**Results**

Statistical analysis showed that NHA had less sealing ability than MTA at the first two periods (day 3, 9), while no significance difference was observed between MTA and NHA at the rest of the study periods in both non-obturated and obturated root canals. It was also found that significant increase in the numbers of bacterial leakage count of failed samples observed with non-obturated than obturated groups for MTA and NHA Fig. (4).

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**Fig. (1): Distribution of specimens through groups and subgroups.**

**Fig. (2):** Manitol salt broth in the left eppendorf tube with no evidence of color change and clear broth, while in the right one show yellow broth color change with turbidity.

**Fig. (3):** Spectrophotometer used in the study adjusted at a wave length of 450nm.
Discussion

The primary aim of root canal treatment is the elimination and future exclusion of all microorganisms from the root canal system. However, if conventional root canal treatment is impossible or has failed, an alternative approach will be necessary. Periapical surgery which entails apicectomy and retrograde root filling may be performed [1].

Several bacterial species were used in bacterial leakage methods in in-vitro studies as; staphylococcus salivarius [2], staphylococcus epidermidis [3], serratiamarcescens [4], and enterococcussfaecalis [5].

Staphylococcus aureus is a facultative anaerobic bacterium isolated from failed endodontically treated root canals [6]; therefore it was used in this study. Also, it is easily detected by the production of acidic environment which rapidly convert the low PH of the growth media because fermentation of manitol lead to detectable color change that enable micro leakage to be seen easily at measuring period of the study.

Other reason is that staphylococcus aureus bacteria have a selective growth media (manitol salt broth), used in this study, to reduce the probability of bacterial contamination with other bacterial spices, which is considered the main limitation of using bacterial leakage model system.

Numerous materials have been used for root-end filling including amalgam, gutta-percha, reinforced zinc oxide eugenol cement, glass ionomer cement, and Mineral Trioxide Aggregate (MTA) [7].

The choice of a retrofilling material could be governed by several properties including biocompatibility, apical sealing ability, handling properties and long term clinical success.

Sealability studies are important in endodontics, especially as an initial screening for newly developed filling materials.

MTA is ranked with good sealability results in several studies [8]. It is important that new tested endodontic materials used as retrograde cavities filling display at least similar ability to prevent leakage as MTA [9].

MTA have shown that calcium and phosphorus are the main ions present in this material. These are also the principal ions of dental hard tissues; therefore it is biocompatible when used in contact with cells and tissues [10].

Hydroxyapatite (HA) is one of the bioceramic materials that forms the principal mineral component of bone and comprises 60% to 70% of the calcified skeleton. It has an excellent biocompatibility with hard tissues [11].

The appearance of nanotechnology which is the study of the controlling of matter on an atomic and molecular scale, improve the properties and sealability of endodontic materials used as the active nanoparticles can penetrate the dentinal tubules and enters the accessory canals to ensure that the spaces have been sealed effectively. Thus in the present study, nano hydroxyapatite was examined for their sealing ability against internal apical bacterial leakage.

It was found that no significance difference was observed between MTA and NHA in both non-obturated and obturated root canals. This may be related to that MTA dissolved leading to the formation of hydroxyapatite crystals, which reacted with dentine to create a chemical adhesion. The fact that bioceramic cements (NHA) contain hydroxyapatite may explain the comparable leakage results to MTA observed in the present study. Also, the HA nanoparticles can effectively seal the exposed dentinal tubules [12].

It was concluded that the NHA provide acceptable sealing ability against bacterial leakage, which was in agreement with several studies examined the sealing ability of HA when used as root-end filling material [13].

There was significance difference between non-obturated and obturated groups for MTA throughout the study periods. Also, between non-obturated...
and obturated group for NHA with better sealing ability against apical bacterial leakage to obturated groups with both tested materials used. Also, the significant increase in the rate of bacterial leakage count of failed samples was observed at day 30 with non-obturated group, while in obturated group observed in latter time period at day 60 when MTA and NHA used as retrograde filling material. That confirms the importance of obturation of the root canals after complete cleaning and shaping with gutta-percha and endodontic sealer which provide helpful sealing on infected dentinal tubules against internal apical bacterial leakage in surgically endodontically retreated cases [14].

Conclusion:
Both bioceramic MTA and NHA provide good apical sealing when used as a retrograde filling material, so NHA could be used as an alternative to MTA, being more cheap and available than MTA.

References

الملخص العربي

تكون معظم حالات علاج فوائد الجروح الفاشلة تكون نتيجة التسبب البكتيري من داخل الفوائد الجلدية، التدخل الجراحي للوصول إلى الجزء المصابة من الجروح يزيد الحساسية الخلية لإحكام غلق الفوائد الجلدية يساعد على زيادة فرصة نجاح العلاج وبالتالي الحفاظ على السن.

هدف الدراسة: تقييم قدرة كلا من المواد مينزال تراي اوكيسيد اجريجات والثاني هيدروكيسي اباثيد علىمنع التسبب البكتيري من داخل الفوائد الجلدية المحلية حيث استخدمها كحشوات جلدية خفيفة.

المواضيع والطرق: مانعة شرسة وحيدة الجروح مخلوسة حديثا تم جمعها لهذه الدراسة. تم إزالة تيبان الأسنان ثم تحضير قنوات الجروح باستخدام مياريد بدوي، بعد ذلك تتعمق الجروح في جهاز التغذية. تم إدخال 10 ميكروليتر من المانع البكتيري النقي والمجهز مسبقا إلى قنوات الجروح باستخدام أنبوب دقيق، ثم توضع الجروح في أحماض أبيندورف معقولة وتحزن عند درجة حرارة سبعة وثلاثون درجة مئوية لمدة ثمان واربعون ساعة.

توزع العينات على المجموعتين: تجريبيا وعددها ثمانيون عينة واسترشادية (تحكيم) وعددها عشرون عينة. تقسيم العينات التجريبية إلى قسمين:

أ- الفوائد الجلدية غير مخصوصة (فارغة).

ب- الفوائد الجلدية مخصوصة باستخدام (جتا بيركا + سيلر). بعد ذلك يقطع الجروح من نهايته بشكل عمودي، ثم تخصص الفوائد الجلدية بالفناء الجلدية الخفيفة.

ثم تقسيم بعد ذلك كل مجموعة من المجموعتين التجريبتين (أ، ب) إلى قسمين تتوافقين على حسب المادة الخشوية الجلدية الخفيفة للمستخدمة. يحتوي كل منها على عشرون عينة. أما المجموعتين الاسترشادية فتقسم إلى قسمين مشابهين (سلاوية ويجابية) كلا منهما يحتوي على عشر عينات. يتم تثبيت العينة داخل أنبوب أبيندورف معقولة تحتوي على محلول بيلي مغذي للبكتيريا وبعد ذلك يتم غلقها، ثم توضع بشكل عمودي داخل حامل الأنبوب وتحزن عند درجة حرارة 37 درجة مئوية وملحة الأنابيب عند الفترات الزمنية طوال تسخين يوم، عند كل فترة زمنية يتم تقسيم أعداد البكتيريا المشردة للعينات التي تظهر لونا أصفر فاتح ومكتوبة محلول البيئي داخل أنبوب أبيندورف.

النتائج: يظهر التحليل الإحصائي لنتائج التجريبية أن لا يوجد اختلاف جوهري بينهما سواء كان الفوائد الجلدية محضرة أو غير محضرة.

اعتماد البكتيريا المشردة من الفوائد الجلدية غير محضرة تكون أكثر كثافة من ووجودها من الفوائد الجلدية المحضرة سواء باستخدام الثانوي هيدروكيسي اباثيد أو المينزال تراي اوكيسيد اجريجات كحشوات جلدية خفيفة.

الخلاصة: يمكن استخدام الثانوي هيدروكيسي اباثيد كديل مقبول عن المينزال تراي اوكيسيد اجريجات حيث أنها أكثر توافرا وأرخص شمًا.