

The Effect of chlorhexidine irrigation on root dentin microhardness in regenerative endodontics using double antibiotic paste

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Method Article

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Abstract

Immature teeth with necrotic pulp represents a challenge in Endodontics management owing to thin fragile roots and blunderbuss apices . Clinically, multiple visit Apexification with calcium hydroxide or one visit Apexification with Mineral trioxide aggregate (MTA) is preferred by clinicians however it has several disadvantages including long term period and increased root dentin brittleness, increasing risk of root fracture

Root canal irrigants and intra-canal medicaments used during Endodontic regeneration procedure may negatively affect the physical and mechanical properties of radicular dentin. Long term exposure of dental hard tissues to acidic antibiotics might cause demineralization and negatively affect their mechanical properties

Matrix Metalloproteinases (MMPS) are proteinases that participate in the degradation of the extracellular matrix (ECM).Chlorhexidine (CHX) is a potent Matrix Metalloproteinases (MMPS) inhibitor, may inhibit the catalytic activity of MMPs.CHX prevents and decreases the release of MMPs by its protein inhibition action (Proteinase inhibitor). Thus we can presume that the increase in the micro-hardness may be attributed to the better preservation of the collagen fibrils of the dentinal surface after treatment with two percent CHX.

Introduction

Immature teeth with necrotic pulp represents a challenge in Endodontics management owing to thin fragile roots and blunderbuss apices (1). Clinically, multiple visits Apexification with calcium hydroxide or one visit Apexification with Mineral trioxide aggregate (MTA) is preferred by clinicians (2). Although multiple visits Apexification with calcium hydroxide is successful , it has several disadvantages including long term period and increased root dentin brittleness with increasing risk of root fracture (2). One visit Apexification by placement of an apical barrier using MTA is an alternative that can shorten the treatment period (2).

However, these approaches did not reinforce the thin and weak roots of immature teeth with the existing risk of root fracture, as the root width does not increase (1, 2). Endodontic regeneration (ER) has been used in the last decade as a treatment option to manage the necrotic immature teeth (2). In this treatment procedure, the root canal is not cleaned mechanically to its full length and hence, root canal disinfection using intracanal medicaments is an essential requirement for endodontic regeneration (2, 3).

Currently, there are three antimicrobial formulations recommended by the AAE for the disinfection of immature permanent teeth with necrotic pulps: CA (OH)₂ , traditional TAP, and modified TAP formulations, with the latter comprising a mixture of three antibiotics lacking in minocycline (MINO), which should be

replaced by an alternative antibiotic to prevent tooth discoloration, or a mixture containing only two antibiotics different from MINO (Double Antibiotic Paste) (4).

Using acids in Endodontics can have a clinical benefit as an irrigation solution for removing the smear layer and for providing a softening effect on dentinal walls, which facilitates rapid preparation and the negotiation of narrow root canals. Antibiotic pastes are acidic in nature (PH =2.9 for TAP and 3.4 for DAP). Acids are commonly added to maintain chemical stability, control tonicity or to ensure physiological compatibility (1- 4).

Triple antibiotic paste (TAP); a mixture of metronidazole, ciprofloxacin and minocycline is the most widely used intra canal medicament in Endodontic regeneration. These three antibiotics provide antimicrobial activity against most endodontic pathogens (1- 4).

Long term exposure of dental hard tissues to acidic antibiotics might cause demineralization and negatively affect their mechanical properties. Minocycline has been found to chelate calcium and demineralize dental hard tissues (1-4).

Root canal irrigants and intracanal medicaments used during ER procedure may negatively affect the physical and mechanical properties of radicular dentin. The use of NaOCL, EDTA, and antibiotic paste was found to significantly reduce dentin flexure strength, microhardness and root resistance to fracture. Changes in the mineral content ratio may reduce the microhardness and increase the permeability and solubility of the root canal dentin. As demineralized dentin is structurally non-supportive, it is essential that the microhardness of this tissue is retained (1, 2, 4).

In the majority of ER cases, the increase in root wall thickness was found to be limited to middle and/or apical root structures rather than cervical part of the root. Therefore, avoiding long term use of antibiotic medicament can decrease the potential for further weakening of cervical area prone to fracture in treated immature teeth with necrotic pulps(4).

Previous studies were conducted using various concentrations of antibiotic pastes and substituting TAP by DAP or MTAP in an attempt to minimize the reduction in microhardness and to avoid crown staining (5).

Current protocols suggest the use of double antibiotic paste (DAP) composed of metronidazole and ciprofloxacin combination. DAP has been used successfully in ER substituting TAP to avoid crown discoloration due to minocycline (5) and it was found to have comparable antibacterial effects against endodontic pathogens (6).

The clinical ER procedure suggested by the American Association of Endodontists (AAE) recommends the use of 1.5% NaOCL irrigation in first visit (20 mL/canal, 5 min) then irrigated with saline (20 mL/canal, 5 min), with irrigating needle positioned approximately 1 mm from the root end. Such low concentration of NaOCL is used to avoid toxic effects on stem cells. Then, the canals are dried with paper points and application of antibiotic low concentrations paste to avoid detrimental effects on stem cells (1–10

mg/mL) for 2 - 4 weeks. In the next second visit and upon the confirmation of the absence of clinical signs and symptoms, irrigation with 17% Ethylenediaminetetraacetic acid (EDTA) (30 mL/canal, 5 min) is recommended to release growth factors from dentin and promote stem cells survival and differentiation, as well as to neutralize the cytotoxic effects of NaOCL and intracanal medicament (7). This is followed by induction of bleeding to produce natural scaffold and deliver stem cells into the canal.

Matrix Metalloproteinases (MMPs) are proteinases that participate in the degradation of the extracellular matrix (ECM) (8). They constitute a large family of zinc and calcium dependent endopeptidases (8). Few MMPs function constructively during remodeling tissues and mineralization of dentin, meanwhile other MMPs can have an adverse effect on dentin (9).

These deleterious enzymes are shown to be released after the exposure of dentin to acids and further break down the collagen matrix and degrade them. This, in turn, results in reduced bond strength and microleakage (9).

Chlorhexidine (CHX), a potent Matrix Metalloproteinases (MMPs) inhibitor that inhibit the catalytic activity of MMPs by binding Zn^{2+} or Ca^{2+} . Thus, it can reduce the collagen degradation by inhibiting host derived proteases in demineralized dentine. The effect of MMPs inhibition increases as the concentration of CHX becomes higher (10).

Chlorhexidine (CHX) has been studied as an irrigant and intracanal medication. It was very effective in eliminating *E.faecalis* (11) with promising results in revascularization therapy (12). CHX has substantive antimicrobial activity depending on the concentration not on its mode of application (11-12). Due to its cationic nature it can be adsorbed onto hydroxyapatite and teeth. At higher concentrations (> 0.02%), a multilayer of CHX is formed on the surface providing a reservoir that can release excess CHX if its concentration decreases (11-12).

Two percent Chlorhexidine in liquid form had a similar microbial performance against several microorganisms as 5.25% sodium hypochlorite. However, it is not a tissue dissolving agent. CHX solution (2%) inhibited bacterial growth after a contact time of only 15 seconds (13).

Various studies have demonstrated the preservation of hybrid layers with CHX treatment after acid etching. CHX prevents and decreases the release of MMPs by its protein inhibition action (proteinase inhibitor). Consequently, we can presume that the increase in the microhardness may be attributed to the better preservation of the collagen fibrils of the dentinal surface after treatment with two percent CHX (11, 13).

Chlorhexidine may have direct and indirect effects on stem cells. The direct effect occurs when CHX is extruded beyond apex and comes in contact with stem cells. However, stem cells are encapsulated in the papilla tissue compared to the in vitro situation. In a clinical protocol, Limitation of the contact time less

than five minutes and a thorough rinse with saline are necessary to provide an environment where stem cells of the apical papilla (SCAPs) can resist and survive (14).

The indirect effect due to its substantivity on dentinal walls, when stem cells come in contact after bleeding induction. Interestingly, the indirect cytotoxicity of CHX was neutralized by a subsequent rinse with L alpha lecithin presumably because of binding of the cationic CHX to the negatively charged scavenger molecule. The final irrigation with EDTA, as recommended for regenerative procedures, did not alter its neutralizing effect (14).

Up to this date there was no study that can correlates the effect of chlorhexidine irrigation on microhardness of root canal dentin wall in Endodontic regeneration procedures using Double antibiotic paste which is the aim of this in vitro study.

Reagents

Equipment

Procedure

Freshly extracted human single rooted teeth will be collected from oral surgery clinic.

Inclusion Criteria:

- 1- Completely formed apices.
- 2- Straight roots or slightly curved with angle ranging from 0 to 10 degrees according to Schneider method
- 3- Single canal

Exclusion criteria:

- 1- Endodontically treated teeth.
- 2- Fractured or cracked teeth.
- 3- Carious teeth.

Debris and soft tissue remnants on the root surfaces will be removed using a scaler. The teeth will be stored in Normal saline at room temperature and will be used within 6 months after extraction

Intervention for each group

Preparation of Samples:

A) Access and instrumentation of the root canals

The teeth will be decoronated at the cement enamel junction with a water cooled high speed diamond bur, leaving a standardized 14mm \pm 1mm length of root samples.

The root samples will be randomly divided into two groups according to irrigation protocol.

1) The intervention group :

Before chemo mechanical preparation, a Two mm of cervical radicular part will be cut off with diamond bur in transverse section, leaving 12mm root length, and then cut it longitudinally into two equal halves to determine the microhardness of root canal wall (pretreatment microhardness) to be as a standard comparison for the intervention group.

Apical patency of the remaining root sample will be checked with size 15 K file (MANI,INC. Japan) Until the tip will be just visible in the apical foramen and subtracting one mm from the length of the file to determine the working length.

Each root canal will be prepared using Rotary Nickel Titanium system M3 pro gold (United Medical Group, China) in crown down technique till size 40 taper four or until clean white chips of dentin is observed under constant speed and torque of manufacturer instructions, using a gentle in and out motion. NaOCL (1.5%) will be used as an irrigation between every subsequent file, with 30 gauge plastic syringe needle.

After complete instrumentation, irrigation with NaOCL 1.5% (20 mL, 5 min) and then irrigated with saline (20 mL, 5 min) with irrigating needle positioned approximately one mm from root end, Followed by Chlorhexidine two percent Liquid irrigation for < 5 minutes.

Upon completion of the Endodontic preparation, 1mg/mL DAP will be delivered to canal system and left for two weeks.

preparation of 1mg/mL DAP :

A mixture of equal powder portions (1:1) of (ciprofloxacin 250mg) and (metronidazole 400mg), A 100mg of this mixture will be mixed with 100 mL sterile water , then a methylcellulose vehicle will be added to obtain a homogenous mix , as described by Hoshino et al. (1,2).

Low concentrations of antibiotic paste is usually in a liquid form , therefore a methylcellulose vehicle ,as described in previous studies (1) , will be loaded to obtain a clinically applicable antibiotic medicament that can be injected.

After two weeks, the antibiotic paste will be removed by copious saline irrigation for 5 minutes, then irrigation with 17% EDTA (30 mL, 5 min) and a final flush with saline (5 mL, 1 min).

All samples will be closed by temporary filling at the end of preparation.

2) The control group:

Before chemo mechanical preparation, a Two mm of cervical radicular part will be cut off with diamond bur in transverse section, leaving 12mm root length, and then cut it longitudinally into two equal halves to determine the microhardness of root canal wall (pretreatment microhardness) to be as a standard comparison for the control group.

Chemo mechanical preparation will be done using Rotary Nickel Titanium system M3 pro gold (United Medical Group, China) with 1.5% NaOCL irrigation as an irrigation between every subsequent file, with 30 gauge plastic syringe needle.

After complete instrumentation, irrigation with NaOCL 1.5% (20 mL, 5 min) and then irrigated with saline (20 mL, 5 min) with irrigating needle positioned approximately one mm from root end.

Upon completion of the Endodontic preparation, 1mg/mL DAP will be delivered to canal system and left for two weeks.

After two weeks, the antibiotic paste will be removed by copious saline irrigation for 5 minutes, then irrigation with 17% EDTA (30 mL, 5 min) and a final flush with saline (5 mL, 1 min).

All samples will be closed by temporary filling at the end of preparation.

B) Preparation and sectioning of root specimens for post treatment microhardness test (9).

Cervical sections of the two groups, cut previously, will be longitudinally equally sectioned into two halves and one section will be mounted on resin blocks to determine pre microhardness of root canal wall.

Remaining radicular part of the two groups after removal of temporary filling will be sectioned longitudinally into equal halves:

I) One longitudinal section will be taken from each of the two groups to be mounted on acrylic resin blocks, leaving their dentin exposed to facilitate the measurement procedures

II) The root canal dentin wall surfaces of the mounted specimens will be grounded flat and smoothed by discs and Silicon carbide abrasive papers under distilled water to remove any surface scratches and finally polished with the composite polishing kit.

C) Post treatment microhardness tests after two weeks (9).

The microhardness of the specimens will be measured using Vickers microhardness tester.

The indentations will be made with a Vickers's diamond indenter onto root canal dentin wall at three levels cervical, middle and apical.

The mean value of the three levels will be calculated for all the specimens and expressed as Vickers Hardness Number.

Troubleshooting

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