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ROLE OF CHELATING AGENTS IN REGENERATIVE ENDODONTICS : A COMPREHENSIVE REVIEW

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ABSTRACT

The ultimate goal of both vital pulp therapy and regenerative endodontic procedures is to completely regenerate the dentin-pulp complex, both structurally and functionally. Ideally, the goal is to regenerate a vital dental pulp covered with dentin to seal the reinfiltration of pathogens. Various irrigants and materials are being used in these procedures. Chelating agents such as ethylenediaminetetraacetic acid play a significant role in these procedures and adds to their successful outcome.

Key Words: Vital pulp therapy, EDTA, Regenerative endodontics

INTRODUCTION

The term chelation is derived from the Greek word “chelos” or “claw” which refers to the mineral- or metal-binding properties of certain compounds that can hold a central cation in a pincerlike grip.[1] In 1957 Nygaard and Ostby introduced chelating agents in endodontics as an aid to prepare narrow and calcified canals. They recommended the use of 15% ethylenediaminetetraacetic acid (EDTA) solution which was thought to chemically soften the root canal dentine and dissolve the smear layer, as well as increases dentine permeability.[2] Since many new chelators have been introduced, a lot of research has been conducted on different types of chelating agents and their various properties and applications, the most recent being regenerative procedures.

Regenerative endodontic techniques are biologically based operations that aim to restore damaged tissues such as dentin and root structures, as well as pulp-dentin complex cells. In 1952 Dr. B W Hermann in a case report mentioned the application of Ca $(OH)^2$ in vital pulp amputation. Since then, much research has been conducted in this direction giving rise to effective vital pulp therapy and revascularization procedures.[3]

This article aims to discuss the role of chelating agents in regenerative endodontics procedures

DISCUSSION

In immature permanent teeth with necrotic pulps, regenerative endodontic therapy causes dentinal walls to thicken and root formation to continue. The protocols which are followed for regeneration in the root canal is: (1) disinfection of the root canal system, (2) bleeding introduced into the canal to provide a scaffold for tissue ingrowth, and (3) mineral trioxide aggregate for coverage. Intracanal disinfection is the first step and a crucial prerequisite for regenerative therapy, which is achieved by using various irrigants and medicaments.[4]

Sodium hypochlorite (NaOCl) is most commonly used for this purpose in regenerative therapy. However, some authors have revealed that treating root canal surfaces with NaOCl reduces the attachment and survival rate of stem cells because of its toxicity. Many investigations have indicated that NaOCl causes dentine resorption and it destroys proteins on the dentine surface as well.[5]

On the contrary, chelating agents like EDTA promote the survival of the stem cells and intimate adhesion of stem cells on the dentin.[4] EDTA treatment results in a close association of cells with the dentine wall as well as cell differentiation. EDTA also leads to release of growth factor on the dentin surface.

MECHANISM OF ACTION

EDTA is a very potent chelator that binds and removes calcium ions from hydroxyapatite, causing the mineral phase to break down. Several growth factors, including transforming growth factor-b1 (TGF-b1), fibroblast growth factor-2, and others, have been found in the EDTA-soluble portion of demineralized human dentine extracellular matrix. These chemicals influence, angiogenesis, immunodefence, cell recruitment, differentiation, proliferation and mineralization even at extremely low concentrations and trigger cellular response.[3]

When utilized as the final step before starting a regenerative endodontic therapy, EDTA irrigation has a positive effect on the behaviour of these cells and the development of new mineralized tissue.[6]

Firstly to begin with, chemotactic substances encourage cell migration into the root canal and draw cells to the dentine surface. Galler *et al* in a study observed chemotaxis towards human dentine after EDTA conditioning, whereas NaOCl pre-treatment showed an adverse effect on cells and untreated dentine did not induce cell migration. Secondly, cell adhesion to the root canal's dentine walls is crucial. According to studies NaOCl treatment hinders cell attachment, but EDTA conditioning allows for adhesion similar to that of untreated dentine. Furthermore, even after three days, EDTA treatment allows pulp cells to proliferate on dentin. Thirdly, cell differentiation is required for the cells to develop a mineralized matrix and complete root formation. Gene expression has shown an increase in mineralization associated markers that have been tested for cell grown on EDTA-pre-treated dentine compared to untreated dentine. Thus, cell differentiation towards a mineralizing phenotype appears to be aided by EDTA treatment.[5]

To reverse the effect of NaOCl and harness the bioactivity of dentine by releasing growth factors, Galler *et al*. advised the use of EDTA on dentine in the pulp chamber or root canal during treatments such as pulp capping, pulpotomy, or revascularization.[6]

Vital pulp therapy procedures keep the dental pulp vital and stimulate the residual pulp to repair the dental-pulp complex. Although there is agreement on the best pulp dressing materials, the recommended irrigant for VPT in permanent teeth receives less attention. Irrigants for root canal treatment should remove all vital and necrotic tissues, microorganisms, and their by-products from the root canal system, whereas irrigants for VPT should have an antimicrobial effect and be biocompatible to aid in the preservation of vital tissues and the vitality of the tooth. They should be bioactive and capable of stimulating the pulpal repair process, which is VPT's primary purpose.

Other minor features, such as not creating detrimental effects on the remaining tooth structure, restoration bond strength, or any discolouration of the tooth after treatment, can all help to improve the quality of VPT.[7]

NaOCl has some unwanted properties for VPT such as cytotoxicity, detrimental effects on remaining tooth structures and bond strength of restorations and it may also cause tooth discoloration when it is used in conjunction with Calcium silicate-based cements.

On human pulp cells, an in vitro study compared 0.04 percent, 0.08 percent, 0.16 percent, and 0.33 percent NaOCl to physiologic saline buffer in terms of cytotoxicity. When the concentration and exposure duration of NaOCl were reduced, cell viability increased, but at concentrations of 0.16 percent and 0.33 percent NaOCl, cell viability declined significantly.[8] Another study by Rosenfeld et al. found that beside necrotic tissues,5.25% NaOCl also affects healthy vital pulp tissue.[9] Furthermore, predentin, or unmineralized dentin with collagen fibers, nerve fibers, and odontoblastic processes, was dramatically reduced in nearly all NaOCl-treated teeth, compared to the normal saline group, which exhibited predentin in all specimens. According to a systematic review there is strong evidence which suggests that NaOCl has adverse effects on the tooth structure.The alteration of tooth structure by NaOCl can also influence the interaction between the tooth surface and restorative material.[10] However, some studies have shown controversial results on the effect of NaOCl on bond strength. It could be owing to its oxidising property, which, together with the irrigant's residual chemicals within the dentinal tubules, interferes with resin composite polymerization.[11] Furthermore, the usage of NaOCl causes the dentin to lose organic content, resulting in an uneven hybrid layer. Reduced bond strength can have a negative impact on the quality of the coronal restoration, resulting in microleakage that could jeopardize the VPT outcome. When NaOCl is used along with MTA, discoloration of the VPT-treated teeth has been observed in both in vitro and clinical investigations. The reaction between NaOCl and bismuth oxide, a radiopacifier found in MTA, is thought to be the cause.

However, EDTA's bioactive property is observed to be more favorable for treatments with a more biological approach such as regenerative endodontics and VPT. Transforming growth factor betas (TGF-βs) are plays a critical role in regulating mesenchymal cells for dentinogenesis. An in vitro study found that irrigation with 17 percent EDTA resulted in much more TGF-1 release than the negative control and NSS, as well as significantly higher cell survival than the 37 percent phosphoric acid group.[12] This bioactive effect of EDTA can promote pulp repair and regeneration, which is necessary for healing and maintaining pulp vitality following VPT. The biocompatibility of 17 percent EDTA was demonstrated in an in vitro investigation, where it surpassed 25 percent tetracycline solution, 5.25 percent NaOCl, 10 percent citric acid, and NSS in terms of DPSC attachment and cell survival.[13]

Furthermore, 17 percent EDTA is non-toxic to cells and increases cell proliferation by improving cell attachment by removing smear layers.Another study also demonstrated that EDTA induced cell attachment and odontoblastic/osteoclastic differentiation of the DPSCs. Also, 17 percent EDTA increased the proliferation and viability of apical papilla stem cells, but NaOCl had the opposite impact in a dose-dependent manner, with a significant drop at a concentration of 6 percent NaOCl. Furthermore, EDTA had much lower cytotoxicity than NaOCl and CHX in an in vitro investigation, indicating that it is a more biocompatible solution than NaOCl and CHX.[14] Bahcall *et al.* advised using 17 percent EDTA in VPT after cryotherapy (sterile-water ice at 0°C) instead of NaOCl.[15] In a clinical trial, MTA was employed as a pulp dressing material for partial pulpotomy, and autogenous treated dentin matrix (TDM) was created by soaking and treating dentin chips from one of the patient's own third molars in EDTA. Although there was no significant difference in clinical or radiographic outcomes between the two groups after 6 weeks, the MTA plus TDM group had much

more dentin bridge development.[16] The bioactive qualities and biocompatibility of EDTA were demonstrated to be effective in the treatment of VPT in this investigation.

Unfortunately, EDTA's chelating ability has some drawbacks as well. In an in vitro investigation, seventeen percent EDTA was found to inhibit blood clot formation. The cause for this was due to its chelation reaction with calcium ions in the blood, which resulted in the blood clot having lower and shorter fibrin networks. Another issue to consider is the interaction between EDTA and calcium ion containing pulp dressing materials. When compared to the distilled water and NSS groups, the results of a study by Lee *et al.* demonstrated decreased crystalline structures and microhardness of the material when subjected to EDTA.[17]

Furthermore, compared to the group stored in distilled water, the EDTA group had decreased cell adhesion on the MTA surface. All of this data revealed that EDTA degrades MTA's characteristics. When EDTA was used with MTA Angelus, there was a substantial difference in colour alteration when compared to specimens in the NSS group, as well as the material in dry condition with no interaction with any irrigant or control group. Another recent study reported that using a spectrophotometer, there was a substantial variation in colour measurement before and after several types of CSCs were immersed in EDTA, but that the difference was undetectable visually.[18] Despite its advantages and promise as a VPT irrigant, there are currently only few clinical investigations utilising EDTA.

EFFECTS OF EDTA ON BLOOD CLOT IN REGENERATIVE ENDODONTIC PROCEDURES

In the regenerative endodontic procedure, bleeding is usually induced from periapical tissues which influxes into the root canal space. A clot naturally occurs after tissue injury by activation of thrombin and fibrinogen to form a cross-linked fibrin network scaffold. Blood clots have been broadly used in the biomedical field as a scaffold for stem cell homing because they consist of essential growth factors to support stem cell proliferation and differentiation.[19]

Blood clotting is the process of forming a natural clot wherein blood changes from a liquid to a gel. It has many advantages over other alternative scaffolds, such as no allergic reaction, reduced costs and visiting time, convenience, and comfort for patients. The clotting process involves many blood cells and clotting factors. One of the clotting factors is calcium ions. Calcium ions as a cofactor play a vital part in the clotting process. They are required for the activation of factors II, VII, IX, X, and platelets. The calcium ions in blood are chelated by remaining EDTA in an EDTA-treated canal, causing the clotting process to be disrupted.

In a study by Peerapohn Taweewattanapaisan *et al* on the effects of EDTA on blood clot in regenerative endodontic procedures, a red blood cell deformity could be observed in the EDTA-treated groups (like shrinkage and crenation caused by water diffusing out of the cells). Stacks or aggregations of erythrocytes, which are called rouleaux formation, were also observed in the EDTA-treated groups. This formation may be associated with factors, such as increased plasma proteins. The blood morphologic characteristics were affected by EDTA as well as fibrin formation in a time-independent manner.[19]

CONCLUSION

Chelating agents such as EDTA should be used on dentine in the pulp chamber or root canal during procedures such as pulp capping, pulpotomy or revitalization to reverse the effect of NaOCl and harness the bioactivity of dentine by release of growth factors. Future studies should focus on the evaluation of different concentrations and time of use for different and newer chelating agents.

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