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Original Article

Effects of using different dentin conditioners on dentin regeneration

Hiwa Saeed Khidir[1]*, Sazan Sherdl Saleem[2]

[1]Department of Dental Assistant, Erbil Medical Technical Institute, Erbil Polytechnic University, Ministry of Higher Education, Kurdistan Regional Government, Erbil 44001, Iraq
[2] Department of Pedodontics, Orthodontics and Preventive Dentistry, Collage of Dentistry, Hawler Medical University, Erbil 44001, Iraq

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Abstract

This experiment aimed to evaluate the impact of several dentine etching and conditioning agents on growth factors (GFs) liberation from dentine slices. Eighteen dentine slices were obtained from nine premolars divided in to six groups, the slices immersed in one mL test solutions for 5 min; Group 1: white Mineral trioxide aggregate (MTA), Group 2: Phosphate buffered saline (PBS), Group 3: 37% phosphoric acid, Group 4: 17% Ethylenediaminetetraacetic Acid (EDTA), Group 5: 10% Maleic acid (MAc), and Group 6: 0.7% Fumaric acid. The solutions were removed and stored directly for further detection and quantification of transforming GF beta 1 (TGF-b1), bone morphogenetic protein 2 (BMP2) and vascular endothelial growth factor (VEGF) by enzyme-linked immunosorbent assay (ELISA). One-way ANOVA was used to compare the mean release and standard deviation between groups ($\alpha = 0.05$). Tukey's post hoc applied for multiple comparisons. After five min conditioning of dentine slices, white MTA released the highest level of TGF-b1, BMP2 and VEGF among all groups, followed by 0.7% Fumaric acid with no significant difference between them, but compared to 37% phosphoric acid and PBS groups significant difference observed, which they released the least amount of GFs amongst all groups. Based on the results of this research the detectable release of TGF-b1, BMP2 and VEGF by 0.7% fumaric acid was comparable with white MTA from dentin slices.

Keywords: TGF-b1, BMP2, VEGF, Fumaric acid, Maleic acid

1. Introduction

The dental pulp, the only soft tissue present in teeth, is composed of extracellular matrix (ECM), interstitial fluid, blood vessels, nerves, fibroblasts, odontoblasts, immune cells, and other biological elements. It is in charge of creating dentin, feeding teeth, communicating sensory data, and providing immunoprotection. Dentin is a highly calcified tissue that lies under the enamel and cementum that surround the tooth pulp and form the pulp cavity. The dental papilla of the tooth germ is the origin of dentin and dental pulp, which together compose the dentin–pulp complex (DPC) and have linked roles during development [1]. The fundamental purpose of odontoblasts, which are formed in the late bell stage of tooth development, is to secrete the ECM and then mineralize them to form the primary dentin. Unlike odontoblasts in the tooth pulp, which generate tertiary dentin in response to physiological or pathological stimuli, including pathogens, secondary dentin is continually put down as a physiological process throughout life. Tertiary dentin may take one of two forms: reactionary, which is structurally comparable to physiological dentin, or reparative, which is poorly structured and mostly tubular with cells retained within the matrix [2–4].

Furthermore, the odontoblasts release different signaling molecules that attach to ECM components and stay embedded inside dentine [5–7]. Human dentine-bound GFs that are released after demineralization include the most abundant, TGF-β1, along with BMP2, Platelet-derived growth factor (PDGF), Epidermal growth factor (EGFR), and placenta growth factor (PlGF). Angiogenesis factors like VEGF and basic fibroblast growth factors (FGFs) are also present [6–8].

By stimulating the release of GFs from human pulp cells and encouraging early dental pulp mineralization, the protein TGF-β1 contributes to dentine regeneration. It is also involved in the expression of bone sialoprotein (BSP), dentin sialo phosphoprotein (DSPP), and TGF-β1 receptor during reparative dentinogenesis [9, 10]. During odontogenesis, BMP2 is a protein that is crucial for odontoblast development and differentiation. Additionally, it plays a part in the growth and regeneration of tooth roots [11].

The VEGF is fundamental pro-angiogenic factor in both physiological and pathological neovascularization, it

* Corresponding author.
E-mail address: hiwa.saeed@epu.edu.iq (H. S. Khidir).
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enhances endothelial cells to ECM degradation, migration and tubule establishment in vitro. In vivo VEGF works as a vascular permeability regulator a crucial role in initiating angiogenesis [12].

Sequestered GFs inside the dentine matrix may be a possible source of cell-signaling molecules to initiate dentine repair in trauma or damage instances. When it comes to inducing cellular responses like angiogenesis, migration, proliferation, and recruitment of new cells during wound healing and repair, bioactive chemicals and GFs are useful even at extremely low, picogram levels [13, 14]. To assist in preventing damage to the viable dental pulp, vital pulp treatment techniques depend on precisely these cellular reactions for dentine regeneration at locations of pulp exposure or the deep cavity bases. The inflammatory response, which is particularly significant in deep carious lesions, may be significantly influenced by dentine matrix proteins [15, 16].

The release of GFs from the dentine matrix through the action of therapeutically employed materials like Calcium hydroxide and MTA has been suggested as a potential role for these materials as essential mediators of reparative reactions in damage conditions [17, 18]. Recent research has demonstrated that phosphoric acid, widely used in dental adhesive systems, can release GFs from dentine discs [19].

It is necessary to acknowledge the limitation of an in vitro examination of GF produced from dentine powder, this process is often performed over a longer period more than hours, and in the presence of protease inhibitors. In the context of vital pulp treatment, direct exposure to and liberation of GFs from the dentine area would be of greater importance. In order to better mimic clinical settings, several researches have looked into the properties of bioactive component exposure and release from conditioned mineralized tissue slices as opposed to powder. These investigations [20–22] have shown the capability of numerous clinically utilized conditioning agents, including EDTA, etidronic acid (HEDP), citric acid and MAc, in release of GFs, as TGF-b1, FGFs and VEGF, from dentine discs. On the other hand, fumaric acid, an inorganic acid, has been successfully used to eliminate the smear layer during root canal therapy experiments [23]. To the best of our knowledge there is no research in the literatures conducted to evaluate the release of transforming growth factor-beta one (TGF-β1), BMP2 and VEGF from dentine slices by fumaric acid.

The results are noteworthy because they demonstrate the release of endogenous GFs originating from dentine, a prerequisite for dental pulp repair and tissue engineering therapies currently being used and may be in the future [24].

The objective of the present study was to assess the impact of different dentine conditioning and etching agents (white MTA, PBS, 37% phosphoric acid, 17% EDTA, 10% MAc, 0.7% Fumaric acid) on release of TGF-b1, BMP2, and VEGF from dentine slices.

2. Materials and methods

2.1. Preparation and conditioning of dentine slices

Nine excised caries-free premolars were taken from the oral surgery center at Hawler Medical University (Erbil, Iraq) with the patient's permission and the university's ethical approval. The soft tissue that was connected was taken out through the process of curetting the root surface using a scalpel blade, while the teeth were preserved in normal saline solution. Subsequently, the enamel and cementum were removed by utilizing a dental turbine equipped with water coolant. Teeth were then sectioned horizontally into 1 ± 0.1mm thick dentine slices (approximately 0.1 ± 0.01 gram in weight). Slicing was done by using a low-speed diamond-edged rotary saw (Micerium S.p.A, Italy) under constant water flow. The scalpel was used to remove the pulp tissue.

Dentine slices (18 slices) randomly assigned to six groups (3 slices per each group) immersed at room temperature in 1 mL test solutions for 5 min; Group 1: white MTA (Produits Dentaires SA, Switzerland) pH 11.7 served as positive control, Group 2: PBS (Thermo Fisher Scientific, US) pH 7.4 (negative control), Group 3: 37% v/v phosphoric acid (Dia Dent, South Korea) pH <1, Group 4: 17% w/v EDTA (Vista Apex, USA) pH 8.5, Group 5: 10% w/v MAc (Thermo Fisher Scientific, US) pH 2.74, and Group 6: 0.7% w/v Fumaric acid (Thermo Fisher Scientific, US) pH 2.7. Following removal, the solutions were TGF-b1, BMP2, and VEGF detection and quantification.

2.2. ELISA for TGF- β1, BMP2 and VEGF release

The quantification of TGF-β1, BMP2, and VEGF was performed in the solutions using the protocols specified by the manufacturer (Human TGF-b1 ELISA Kit, MyBioSource, USA), (Human BMP2 ELISA Kit, MyBioSource, USA), and (Human VEGF ELISA Kit, MyBioSource, USA). These kits included capture antibodies, biotinylated detection antibodies, and avidin HRP. Every sample was examined three times during the experiment, following the instructions that came with the ELISA kits.

2.3. Statistical analyses

At a significance threshold of 0.05, one-way ANOVA analysis was used to compare the mean release and standard deviation of TGF-β1, BMP2, and VEGF between study groups. Pairwise multiple comparisons were performed using a post hoc Tukey's method.

3. Results

3.1. Quantification of TGF-b1 Release from Dentin

Figure 1 shows the results of ELISA-based assays of TGF-b1 released from dentin. The greatest levels of TGF-β1 (210 pg/mL) were released after conditioning with white MTA; this effect was not statistically significant when conditioning with 0.7% fumaric acid (194 pg/mL). In contrast, 17% EDTA and 10% MAc had less beneficial results (166 pg/mL and 161 pg/mL, respectively).

3.2. Quantification of BMP2 Release from Dentin

ELISA data revealed the highest concentration of BMP2 release from dentine slices was in the solution of white MTA agent (788 pg/mL) which was statistically significant with all the groups except 0.7% Fumaric acid. However, the difference between agents (17% EDTA, 10% Maleic and 0.7% Fumaric) were not in significant (542, 426, 601 pg/mL respectively). The BMP2 detected in the 37% Phosphoric acid (260 pg/mL) and PBS (240 pg/mL) groups were significantly lower than all other agents ($P<0.05$) (Figure 2).

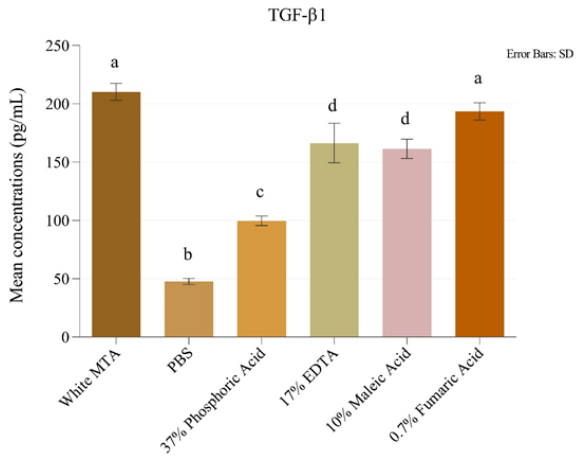

Fig. 1. Mean concentrations of TGF-b1 in the solutions of conditioned dentine. Statistical difference (P<0.05) showed in Compact Letter Display (CLD), Groups without notable differences are indicated with the same Alphabetic small letters (a,b,c and d).

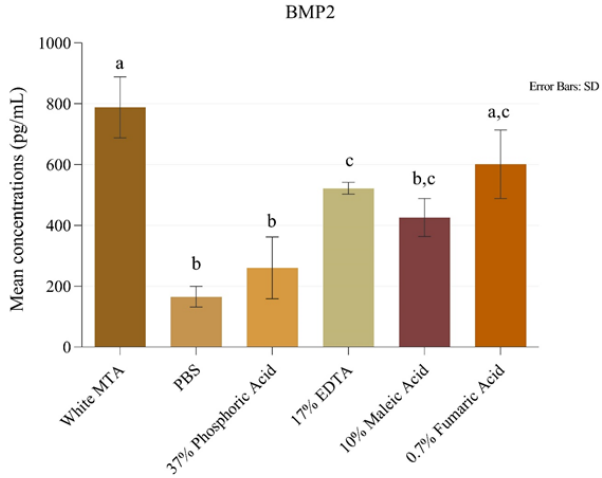

Fig. 2. Mean concentrations of BMP2 in the solutions of conditioned dentine.

3.3. Quantification of VEGF Release from Dentin

After five min of immersion, VEGF was identified by ELISA in the test solution containing all of the agents, as shown in Figure 3. The concentration of the VEGF was highest after conditioning with white MTA (174 pg/mL) and 0.7% Fumaric acid (152 pg/mL) compared with all the other groups. The difference in release of VEGF between 17% EDTA and 10% MAc was not significant (109 pg/mL and 95 pg/mL respectively). Although the difference between 37% Phosphoric acid (44 pg/mL) and PBS (27 pg/mL) was not statistically significant, the concentration of VEGF in these two groups was the least compared with all other groups ($p < 0.05$).

4. Discussion

Dentine is a complicated heterogeneous organization whose organic framework is composed of a range of non-collagenous proteins making up 10%, Collagen makes up about 90% of the matrix. Specifically, throughout dentinogenesis, a number of GFs are retained in the dentine matrix [13].

Dentine repair and regeneration could be enabled by the liberation of TGF-b1 by dental materials or bacterial acids during caries formation and restorative procedures [25]. In combination with other variables, BMP2 has a synergistic effect on reparative dentin development [26]. VEGF role promotes angiogenesis, which is the formation of new blood vessels, in the dental pulp tissue. This angiogenesis is crucial for the delivery of oxygen and nutrients to the regenerating tissue, as well as for the removal of waste products [27].

In this research, dentin was treated for 5 min with various alkaline, acidic, or calcium chelators, as also demonstrated by Atesci *et al.* [19]. Another research looked at various time frames and concluded that it is impractical to increase the exposure period since GFs start to leak after five min. Longer conditioning time increases the likelihood of cell loss necessary for regeneration and dentin erosion, ultimately weakening the immature tooth [19, 20].

Due to the powder's concentration of GFs, the GF freed in prior research using dentin powder was optimized [28]. Similarly, with discs, the discharge occurred from every surface of the specimen. Dentin slices are prepared in an easy-to-standardize manner by immersing them in a conditioner and then measuring the GF.

Our findings demonstrated that dentin treated with white MTA obtained the highest concentrations of three GFs compared to other conditioning agents. This outcome is attributed to the biological and physicochemical properties of MTA, which can stimulate reparative dentin formation through complex cellular and molecular processes involving newly differentiated odontoblast-like cells. MTA's activity is linked to the basic repair process of exposed dental pulps. Additionally, MTA can activate cells that create hard tissues to promote matrix formation and mineralization. Physicochemical investigations of MTA demonstrate its ability to release calcium hydroxide and mix with phosphate-containing fluids to create apatite precipitates [29].

Both fumaric and MAc are mild organic acids, they have also been shown to be less cytotoxic as they showed higher release of GFs than other groups except MTA group, this could be a result of the chemical's pH and the processes involved in protein release. A further possible cause for these findings could be the presence of a more acidic environment that facilitates the denaturation of a peptide linked to the latent TGF-b complex. This denatu-

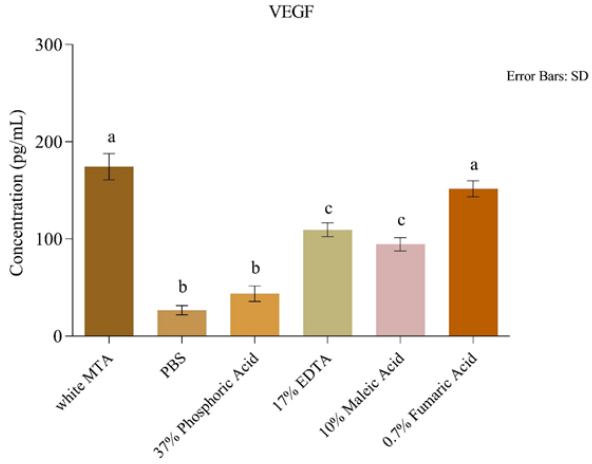

Fig. 3. Mean concentrations of VEGF in the solutions of conditioned dentine.

ration may result in a greater release of TGF-β in its active state, consequently multiplying its biological impact [30, 31]. 10% MAc was found to be with same effective as 17% EDTA in promoting TGF-β1 GF release in contrast with the results found by Ballal *et al.* [21]. The reason for this variation may be the different concentrations of acid which they used 7% MAc and longer immersion time as they quantified GFs after 10 min.

On the other hand, the release of GFs from dentine in the phosphoric acid group was leaser than the maleic and fumaric acid groups, this could be a result of the GF becoming denatured after being released into the solution, as indicated by O'Brien et al. [32]. The consistency and denaturing of the proteins, induced by changes in the surrounding pH, could compromise their detection by means like ELISA once they are released. It has been demonstrated that the kind of acid plays a significant role in identifying how certain proteins perform in an acidic condition, in addition to the effect of pH as indicated by Chae *et al.* [31] reported significant difference between 10% and 37% phosphoric acid concentration effect on TGF-b1 release.

EDTA eliminates the inorganic part of dentine, resulting in a collagen-rich dentine [33], also acid etching demineralizes dentine, leading to a layer with low mineral content but high in proteins [34]. Data from this article revealed that conditioning dentine slices with EDTA significantly releases a greater amount of TGF-b1 than phosphoric acid, the same result reported by Sadaghiani *et al.* [28] and Chae *et al*. [31]. While this result contradicts the findings of Atesci *et al*.[19], as they reported that 37% phosphoric acid significantly released a higher amount of TGF-b1 from dentine, the possible explanation for this is that they used powder of dentine instead of discs. Another cause might be the immersion time, as they used 37% phosphoric acid for 30 sec while in this study the dentine slices were immersed for 5 min.

While TGF-b1 and BMP2 were found to have produced similar results in terms of VEGF liberation from dentin, ELISA found lower levels of VEGF freed from dentin in the current study [19, 20, 28]. These results might be explained by the shorter half-life and decreased amounts of VEGF in dentin [6, 35].

Additionally, the results showed that the levels of VEGF liberated from dentine matrices varied across chemicals. The top agents for releasing these GFs were white MTA and 0.7% fumaric acid, followed by 10% MAc and 17% EDTA (no significant difference between them). This could be a result of the agent's pH along with the processes associated with protein release.

5. Conclusion

Based on the results of this research the detectable release of TGF-β1, BMP2 and VEGF by 0.7 Fumaric acid was comparable with white MTA from dentin slices. Due to its potential to induce the release of GFs, fumaric acid could be a promising choice in terms of biological viability and regeneration as an etching and conditioning agent.

Conflict of Interests

There are no disagreements between the author and any stage of the essay production.

Consent for publications

The author reviewed and gave their approval to the published version of the study.

Ethics approval and consent to participate

The study was approved by the Hawler Medical University's College of Dentistry's Ethics and Scientific Committee.

Informed Consent

The authors state that no patients were utilized in this study.

Availability of data and material

Data are available from the corresponding author upon request.

Authors' contributions

Hiwa S. Khidir and Sazan S. Saleem have given substantial contributions to the conception or the design of the manuscript, Hiwa Saeed Khidir to acquisition, analysis and interpretation of the data. All authors have participated to drafting the manuscript, Hiwa S. Khidir and Sazan S. Saleem revised it critically. Every author has read and given their approval to the completed study. Each author read and approved the manuscript's final draft in addition to making an equal contribution to its creation.

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