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The Effect of Poly Amido Amine Dendrimer, Gluteraldehyde and Their Combination on the Micro Hardness and Micromorphology of Demineralized Dentin

Asmaa Adel Abd-El-Aziz Khater[1], Maha Ahmed Niazy[2] *and* Nevin Abd-El-Aziz Gad[3]

Codex : 40/1810

azhardentj@azhar.edu.eg

http://adjg.journals.ekb.eg

ABSTRACT

Objective: The aim of this study was to evaluate the remineralizing effect of Poly (amido amine) dendrimer, Gluteraldehyde and their combination on demineralized dentin at different time intervals. M ate rials and Methods: A total of one hundred and twenty dentin discs were prepared from extracted teeth (n=120), each dentin sample was immersed in 10% citric acid solution for 30 seconds. The samples were divided into two main groups (60 each) according to the assessment time, one week assessment, and four weeks assessment, These two groups were further subdivided into three groups according to the treatment materials; group (1), (n==10): pure G3.0 PAMAM dendrimer, group (II), (n=10): Gluteraldehyde was applied to the demineralized dentin, Group (III), (n=10): a combination of PAMAM dendrimer and Gluteraldehyde. Each group has its control group (n=10) which didn"t receive any treatment. Each treated group with its corresponding control one was placed in a separate container of artificial saliva for one and four weeks. The samples were subjected to microhardness test and Scanning Electron Microscope (SEM). Resu lts: after one week assessment, the results revealed that the highest mean value recorded for PAMAM group (10.9%) followed by PAMAM Gluteraldehyde group (8.3%) while Gluteraldehyde group recorded the lowest mean value (5.26%). This difference was statistically insignificant. After four weeks assessment, the results revealed that the highest mean value recorded for PAMAM-Gluteraldehyde group (14.77%) followed by PAMAM group (14.34%) while Gluteraldehyde group recorded the lowest mean value (8%). This difference was statistically insignificant Conclusions: All treatment materials used were effective in increasing dentin microhardness and produced micromorphological changes of the dentin surface in terms of occluding the orificies of dentinal tubules to variable degree.

KEYWORDS

Caries, remineralization, PAMAM dendrimer, Gluteraldehyde, SEM, microhardness

INTRODUCTION

Dentin caries and dentin hypersensitivity are common oral diseases, which are both related to the demineralization of dentin.

1. Demonstrator of operative dentistry Department, Faculty of Dental Medicine for Girls, Al-Azhar University, Egypt.
2. Professor and head of Operative Dentisttry, Faculty of Dental Medicine for Girls, Al-Azhar University, Egypt.
3. Lecturer of Operative dentistry, Faculty of Dental Medicine for Girls, Al-Azhar University, Egypt.

The remineralization of dentin, as a non-invasive therapeutic technique in clinical dentistry, is preferred to the traditional invasive substitutes, such as resin and amalgam. [1,2] Dentin is a biomineralized hard tissue composed of hydroxyapatite (HA), organic matrix, and water, [3] and it exhibits complex morphologies and remarkably optimized mechanical properties attributed to the hierarchical arrangement of HA. There is a physiological equilibrium between the remineralization and demineralization of dentin in the oral cavity. The equilibrium will break toward demineralization when the organic acids derived from bacteria or an acidic diet increase. [4]

Demineralized dentin is mainly composed of collagen fibrils, which are unable to initialize HA nucleation and growth.[5] The organic matrix of dentin contains non collagenous proteins (NCPs), which play a very important role in the biomineralization of dentin. A series of NCPs become nucleation templates within the gap zones of collagen fibrils and control the growth of HA during the biomineralization. However, in the mature dentin NCPs lose their abilities to induce remineralization. The remineralization of demineralized dentin requires good nucleation templates that can firmly bind to collagen fibrils. [6]

Poly (amido amine) (PAMAM) dendrimer, often referred to as ''artificial protein'', contains tree-like structures with ''branches'' radiating from one central core. The presence of a large number of external reactive groups in PAMAM dendrimer, its well-defined size and its controlled spatial structure make it suitable for mimicking natural NCPs.[7] it has been reported that PAMAM dendrimers have been widely applied in the biomineralization field. [8] PAMAM dendrimers with different types of functional groups on the external surface such as carboxylic-terminated PAMAM (PAMAM-COOH), amine terminated PAMAM (PAMAM-NH2), and polyhydroxy terminated PAMAM (PAMAM-OH).[9,10] Different generations of PAMAM dendrimers have different structures. The first and the second generations are linear molecules, while the third generation is a sphere molecule and owns more functional groups, which ensure that it could absorb more calcium ions during the remineralization. [11]

Glutaraldehyde (GA) is a gold-standard crosslinking agent that has been widely accepted in the biomedical field and is considered to improve tissue function; it has been revealed that the collagen-based aldehyde-treated tissue has an affinity towards calcium ions, which can act as mineralization centers for calcium phosphate precipitation.[12,13] GA is the main composition of Gluma dentin desensitizer, and 5.0% concentration has less cytotoxic effect.[14] Low concentrations of GA (around 5%) have already been used as a collagen crosslinking agent to enhance the stability of the resin-dentin interface and improve bond durability. [15]

Therefore, this study was designed to evaluate the effect of PAMAM dendrimer, Gluteraldehyde, and their combination on microhardness of demineralized dentin. Moreover the micromorphological changes at the dentin surface were examined using Environmental Scanning Electron Microscope (ESEM) at different time intervals.

MATERIALS AND METHODS

A total of one hundred and twenty dentin discs were prepared from extracted teeth, each with a thickness of approximately of 1.0 mm. The enamel of each tooth was removed first by using diamond disc mounted in cutting machine Demco (Dentallmaintenanc CO, Bonsall, calf. U.S.A, model E96) and then two dentin discs were taken from each tooth by making parallel cuts perpendicular to the long axis of each tooth, one disc served as the control and the other received the treatment material [16] Specimens were embedded in acrylic resin blocks. Each sample was exposed to the acid separately. Each dentin sample was first dried with air way syringe, immersed in 10% citric acid

solution for 30 seconds and rinsed with deionized water for 30 seconds [31] and then the samples were divided into two main groups (60 each) according to the assessment time, one week assessment (T1), and four weeks assessment (T2). These two groups were further subdivided into three groups according to the treatment materials:

In group (1); citric acid treated specimens were single coated with pure PAMAM dendrimer using microbrush for 30 seconds and then rinsed with deionized water for 30 seconds [16] while its control group didn't receive any treatment.

In group (2); citric acid treated specimens were immersed in 5% Gluteraldehyde solution for 3 minutes and then rinsed with deionized water for 30 seconds.[17] while its control group didn't receive any treatment.

In group (3); citric acid treated specimens were immersed in PAMAM-Gluteraldehyde solution for 24 hours and then rinsed with deionized water for 30 seconds [16] while its control group didn't receive any treatment.

Storage of the specimens: Each treated group with its corresponding control one was placed in a separate container of artificial saliva for one week [16] and another similar groups were placed for four weeks at room temperature and the artificial saliva was replenished every 24 hours.

Microhardness assessment:

All the samples were subjected to microhardness assessment after one and four weeks. Surface Micro-hardness of the specimens was determined using Digital Display Vickers Micro-hardness Tester* with a Vickers diamond indenter and a 20X objective lens. A load of 200g was applied to the surface of the specimens for 20 seconds. Three indentations, which were equally placed over a circle and not closer than 0.5 mm to the adjacent indentation, were made on the surface of each specimen. The diagonals length of the indentations was measured by built in scaled microscope and Vickers values were converted into micro-hardness values.

Scanning Electron Microscope:

The surfaces of selected samples were examined using Scanning Electron Microscope (ESM) for evaluating the changes in surface topography; the Specimens were dried and mounted on metal

Statistical Analysis

All the data were collected, tabulated and analyzed. One way ANOVA followed by pair-wise Tukey's post-hoc tests were performed to detect significance between groups. Student t-test was done between material subgroups. Two-Factorial AA was done to detect effect of Variables affecting

RESULTS

MICROHARDNESS:

Comparing the change of mean value of each group after evaluation time 1, the results revealed that the highest mean value recorded for PAMAM group (10.9%) followed by PAMAM-Gluteraldehyde group stubs; images from the selected sample were obtained (at both 2000x and 5000x magnification). (8.3%) while Gluteraldehyde group recorded the lowest mean value (5.26%). This difference was statistically insignificant as revealed by ANOVA test ($F=1.7$, $p=0.1925>0.05$).

Comparing the changes of mean value of each group after evaluation time 2, the results revealed that the highest mean value recorded for PAMAM-Gluteraldehyde group (14.77%) followed by PAMAM group (14.34%) while Gluteraldehyde group recorded the lowest mean value (8%). This difference was statistically insignificant as revealed by ANOVA test ($F=0.8$, $p=0.4662>0.05$).

		Group (AI)	Group (A_2)	Group (A3)	P-value
Evaluation time 1	Change in mean	7.5	3.36	4.17	0.01925*
	(%)	10.9%	5.26%	8.3%	
Evaluation time 2	Change in mean	9.03	4.95	8.9	0.4662*
	(%)	14.34%	8%	14.77%	

**; significant (p<0.05)*

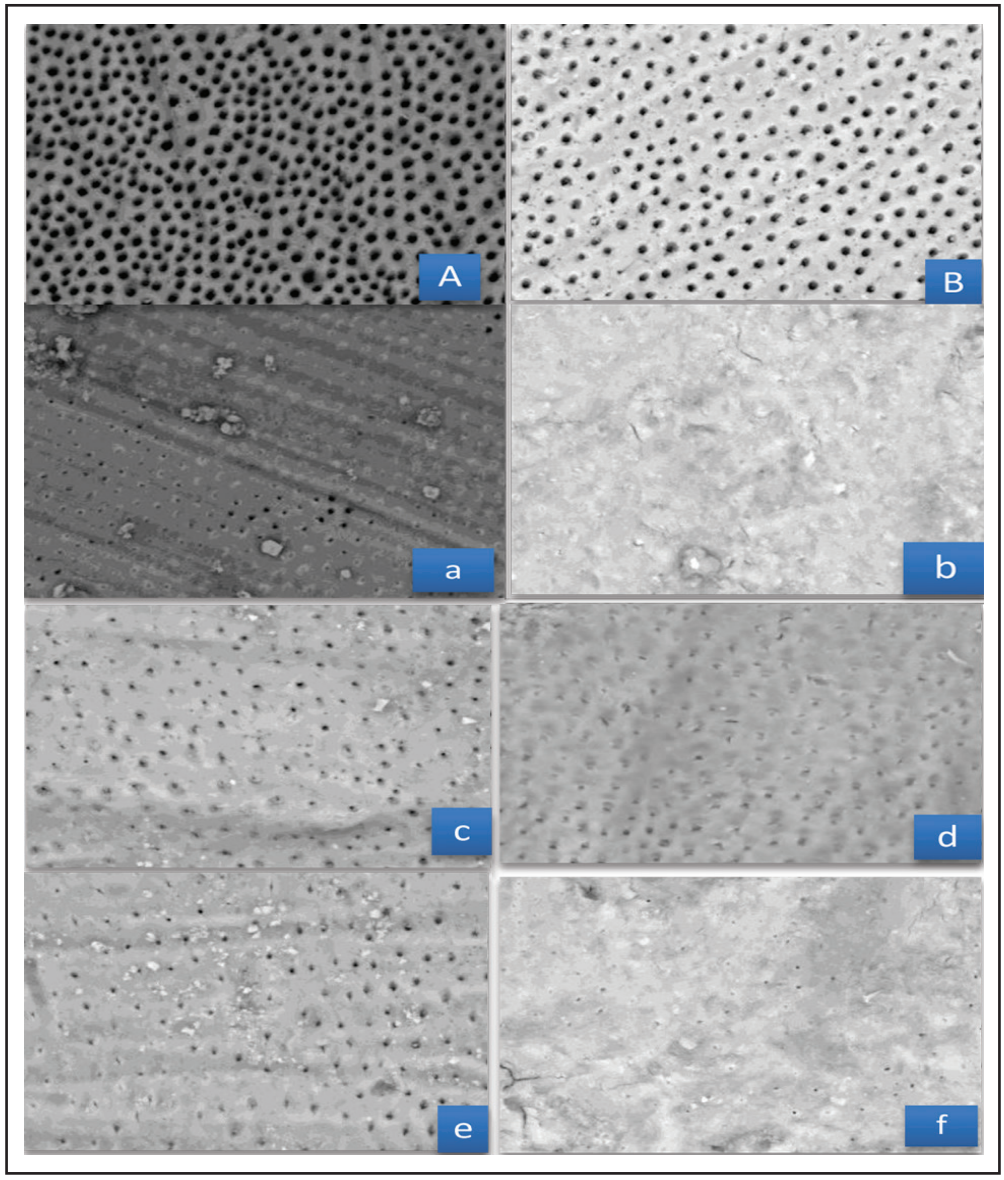

Fig. (1): SEM of dentin surface. (A) demineralized dentin; (B) dentin surface of control group; (a) dentin surface treated with PAMAM after evaluation time 1; (b) dentin surface treated with PAMAM after evaluation time 2; (c) dentin surface treated with Gluteraldehyde after evaluation time 1; (d) dentin surface treated with Gluteraldehyde after evaluation time 2 ; (e) dentin surface treated with PAMAM-Gluteraldehyde after evaluation time 1; (f) dentin surface treated with PAMAM-Gluteraldehyde after evaluation time 2.

Scaning Electron Microscope:

PAMAM dendrimer treated group showed complete occlusion of the opened dentinal tubules, Part of the dentin surface was covered with newly formed precipitates and a very little dentinal tubules were still opened when the specimens were placed in artificial saliva for a week. After four weeks, the dentinal tubules were completely occluded and the dentin surface was covered with newly formed percipitates. In Gluteraldehyde group the opened dentinal tubules were partially occluded with newly formed precipitates when the specimens were placed in artificial saliva for a week whereas after four weeks the dentinal tubules were almost completely occluded. In PAMAM- Gluteraldehyde group the opened dentinal tubules were partially occluded with newly formed precipitates when the specimens were placed in artificial saliva for a week whereas when placed for four weeks most of dentinal tubules were completely occluded while some were almost completely occlude

DISCUSSION

Dentin caries and dentin hypersensitivity are common oral diseases, which are both related to the demineralization of dentin. The remineralization of demineralized dentin requires good nucleation templates that can firmly bind to collagen fibrils. Fluoride is an excellent remineralization agent on enamel, while it is less effective on dentin. In recent years, many research groups have attempted to remineralize demineralized dentin using different nucleation templates such as bioactive glass and Dopamine. These remineralization agents effectively remineralize demineralized dentin to some extent, but the effect of remineralization still needs to be improved.[18,19] In the present study, a promising mineralization inducing agent, the third generation (G3 Poly amido amine) (PAMAM) dendrimer was employed. Poly (amido amine), often referred to as artificial protein, has a large number of external reactive groups, and also has a well-defined size and controlled spatial structure which make it suitable for mimicking natural NCPs.[7] It has been reported that PAMAM dendrimers have been widely applied in the biomineralization field. Gluteraldehyde was used in this study as it has been revealed that the collagen-based aldehyde-treated tissue has an affinity towards calcium ions, which can act as mineralization centers for calcium phosphate precipitation.[17] This precipitation is undesirable in soft-tissue implants but can be an advantage for the remineralization of collagenous mineralized tissues, such as dentin. A concentration of 5% was used as it has less cytotoxic effect.[13]

In this study the crosslinking ability of Glutaraldehyde was employed. PAMAM dendrimers were crosslinked with demineralized dentin collagen using Glutaraldehyde. Free primary amine groups (–NH2) of G3.0 PAMAM dendrimers and collagen fibrils can easily react with the aldehyde groups (-C = O) of glutaraldehyde to form Schiff's base and the two kinds of molecules will be linked with covalent bonding which can also be formed between PAMAM dendrimer molecules and is better than easily interfered electrostatic interaction employed in the applications of other kinds of biomimetic analogs of NCPs (e.g. polyvinylphosphonic acid, PVPA). Surface Micro-hardness (VHN) of the specimens was assessed after demineralization and after the application of treatment materials and storage in artificial saliva for one and four weeks. This assessment method provides relatively a simple, non-destructive and rapid method in determining the mechanical properities of dentin. The micromorphological changes in dentin surface was determined using Scanning electron microscope (SEM) as it gives very detailed image of the surface that enables the study of the changes occurring at the dentin surface. [19,20] In the present study, comparing the percentage change of mean microhardness values of each group after one week, the results revealed that the highest mean values were recorded for PAMAM group followed by PAMAM-Gluteraldehyde group. Gluteraldehyde

group recorded the lowest mean value though the difference was statistically insignificant. After four weeks the highest mean microhardness values recorded for PAMAM-Gluteraldehyde group followed by PAMAM group while Gluteraldehyde group recorded the lowest mean value with insignificant difference between them.

This result of microhardness matched the SEM observations where PAMAM dendrimer treated group showed complete occlusion of the opened dentinal tubules, Part of the dentin surface was covered with newly formed precipitates and a very little dentinal tubules were still opened when the specimens were placed in artificial saliva for a week. After four weeks, the dentinal tubules were completely occluded and the dentin surface was covered with newly formed percipitates. In Gluteraldehyde group the opened dentinal tubules were partially occluded with newly formed precipitates when the specimens were placed in artificial saliva for a week whereas after four weeks the dentinal tubules were almost completely occluded. In PAMAM- Gluteraldehyde group the opened dentinal tubules were partially occluded with newly formed precipitates when the specimens were placed in artificial saliva for a week whereas when placed for four weeks most of dentinal tubules were completely occluded while some were almost completely occluded.

CONCLUSIONS

All treatment materials used were effective in increasing dentin microhardness; with PAMAM dendrimer being the most effective after one week while PAMAM- Gluteraldehyde combination was the most effective after four weeks.

All the treatment materials produced micromorphological changes of the dentin surface in terms of occluding the orificies of dentinal tubules to variable degrees.

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