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Evaluation of chemical and/or mechanical treatments of the smear layer as revealed by scanning electron microscopy – a blind comparative study

Avaliação dos tratamentos químicos e/ou mecânicos da camada de esfregaço conforme observado por microscopia eletrônica de varredura – estudo cego comparativo

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A blind comparative study of chemical and/or mechanical treatments of the smear layer, according to scanning electron microscopy images, was carried out. The effect of the treatments was analyzed on the smear layer of mesio-occlusodistal cavity walls prepared *in vitro* in human third molars. The agents used were air/water spray, 37% phosphoric acid, 5% tannic acid, biologic detergent, 0.5% sodium hypochlorite, and enamel hatchet alone or in association with the previous agents. Electron micrographs were evaluated by three professionals according to the degree of visualization of underlying dentin or enamel. Phosphoric acid received the highest scores due to the complete removal of the smear layer. However, statistical analyses revealed diverse performances of non or slightly demineralizing agents, according to the cavity walls in dentin, while there was equivalent effect on the enamel of gingival walls.

UNITERMS: Smear layer; Dentin; Scanning electron microscopy.

INTRODUCTION

The treatment of the smear layer is an important factor for the good performance of restorations. More resistant unions are obtained when adhesive materials are used, and gaps are minimized when non adhesive materials are used[10,16]. Non or slightly demineralizing treatments are important for the use of adhesive cements, because of their union with calcium and phosphate ions from the cavity walls[24]. Non or slightly demineralizing treatments may be also important for the use of resin adhesives that are applied without removal of the smear layer.

Studies have demonstrated that the formation of the hybrid layer by penetration of monomers into the dentin is more important than chemical adhesion or than the formation of resin tags within dentinal tubules[12]. However, excessive demineralization of dentin gives rise to collagen denaturation, and resins may not penetrate into the matrix as deeply as acidic conditioners do. Thus, a weak zone may be created, with unprotected collagen, causing the failure of adhesion[13,15]. These facts led to the development of adhesives with acidic monomers, i. e. the smear layer may be slightly demineralized due to the association of acids with hydrophilic/hydrophobic monomers. This procedure allows a concomitant diffusion through the underlying dentin, creating a hybrid layer of unaltered collagen involved in resin[12,13,22,23].

Scanning electron microscopy (SEM) is a reliable method to evaluate dentin and enamel in disease or under procedure conditions[4,11]. SEM is also adequate to evaluate the smear layer, although sometimes it is difficult to quantify it. Thus, the

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use of a blind method is valid to evaluate the effect of different treatments on it.

The purpose of this study was to analyze the effect of chemical, mechanical and both treatments of the smear layer, as revealed by SEM, in a blind study, in which the electron micrographs were evaluated by three professionals.

MATERIALS AND METHODS

The study was conducted with thirty fresh, non-carious, unerupted human third molars, obtained from patients between 16 and 27 years of age (mean 21 years). All patients were fully informed about the procedures of this study. Roots were removed, and mesio-occlusodistal cavity preparations executed with new cylindrical diamond burs under water cooling.

Immediately after cavity preparation, one of the following treatments was carried out for each three teeth, being applied on every cavity wall: (1) air/water spray; (2) 37% phosphoric acid, applied with cotton pellets during 15 s; (3) 5% tannic acid, applied with cotton pellets during 15 s; (4) 0.2% lauril sodium sulfate biologic detergent (Tergensol, Inodon), applied with cotton pellets with rubbing during 15 s; (5) 0.5% sodium hypochlorite, with cotton pellets, applied with rubbing during 15 s; (6) enamel hatchet, applied during 30 s; (7) enamel hatchet with 37% phosphoric acid, applied during 45 s (30 s with enamel hatchet alone, plus 15 s with concomitant application); (8) enamel hatchet with 5% tannic acid, applied during 45 s; (9) enamel hatchet with biologic detergent, applied during 45 s; and (10) enamel hatchet with 0.5% sodium hypochlorite, applied during 45 s. Air/water spray was applied for 5 seconds before and after all treatments.

The teeth were longitudinally half-sectioned in a mesiodistal orientation with a chisel. Then, one of the fragments was processed for scanning electron microscopy. Specimens were air-dried and mounted on aluminum stubs. After sputtering with a 40 nm layer of gold in a Balzers SCD 050 apparatus, the treated cavity wall surfaces were examined in a Jeol 6100 scanning electron microscope operating at 10-15 kV.

The electron micrographs were analyzed by three experienced examiners through a blind technique. They graded their observations using a score ranging from 0 to 3, according to the degree of removal of the smear layer and consequent visualization of the underlying dentin or enamel[6]. Five electron micrographs of each specimen were analyzed. Two magnification levels were used for both pulpal and lateral walls (400 X and 2000 X), and one magnification level, for gingival walls (400 X).

RESULTS

Cavity walls treated with air/water spray exhibited variable amounts of smear layer covering the dental walls, with superficial particles not firmly attached to the sound layer. Circular marks produced by the diamond particles of the instrument were evident on the surface of pulpal walls. In general, the depth of the marks roughly indicated the amount of smear layer.

Phosphoric acid treatment showed a complete removal of the smear layer with evidence of the tubular structure of the dentin. Thus, the tubules appeared opened and enlarged, revealing a thin or absent peritubular dentin. In these specimens, the intertubular dentin exhibited a clean and smooth aspect. The demineralization of peritubular dentin caused a funneled-like tubule aspect, showing a decreasing effect of the acid according to the increase in depth of dentin. On enamel of gingival walls, the removal of the smear layer showed the enamel prisms in transverse, oblique or longitudinal sections, depending on the wall level.

Non or slightly demineralizing treatments produced removal of the smear layer in variable amounts. The mean scores related to the performance of the treatments, pertaining to pulpal, lateral, and gingival walls are shown in Tables 1, 2, and 3.

TABLE 1 - Mean scores and standard deviation for efficiency degree of smear layer removal on pulpal walls.

Treatment	Pulpal wall	
	Mean	S. D.
Phosphoric acid	3.00	0.00
EH*+ phosphoric acid	2.95	0.19
EH + tannic acid	0.89	0.46
0.5% s. hypochlorite	0.44	0.17
Tannic acid	0.44	0.40
EH	0.44	0.50
Air/water spray	0.33	0.30
EH + s. hypochlorite	0.28	0.44
EH + b. detergent	0.17	0.28
Biologic detergent	0.17	0.41

*Enamel hatchet.

Analysis of variance and Kruskall-Wallis analysis[7] of the results revealed different effects of non or slightly demineralizing treatments according to the cavity walls in dentin (pulpal walls = 0.0584; lateral walls = 0.0073), and equivalent effect on the enamel of gingival walls (p = 0.8467). Student-Newman-Keuls technique[14] revealed that the treatment with enamel hatchet and 5% tannic acid was more efficient than both biologic detergent treatments on pulpal walls, and that the biologic detergent treatment was more efficient than the one with the enamel hatchet and 0.5% sodium hyphoclorite or 5% tannic acid, on lateral walls (Figures 1, 2 and 3).

DISCUSSION

This study showed that all non or slightly demineralizing treatments of cavity walls produced some removal of the smear layer, although differences between these treatments were quite subtle.

TABLE 2 - Mean scores and standard deviation for efficiency degree of smear layer removal on lateral walls.

Treatment	Lateral wall	
	Mean	S. D.
Phosphoric acid	3.00	0.00
EH + phosphoric acid	2.66	0.35
Biologic detergent	0.78	0.17
Air/water spray	0.56	0.54
Tannic acid	0.56	0.46
0.5% s. hypochlorite	0.28	0.39
EH + b. detergent	0.28	0.33
EH	0.22	0.34
EH + s. hypochlorite	0.11	0.27
EH + tannic acid	0.00	0.00

TABLE 3 - Mean scores and standard deviation for efficiency degree of smear layer removal on gingival walls.

Treatment	Gingival wall	
	Mean	S. D.
Phosphoric acid	3.00	0.00
EH + phosphoric acid	2.33	0.58
Tannic acid	0.89	0.19
Air/water spray	0.67	0.67
Biologic detergent	0.56	0.51
EH + s. hypochlorite	0.56	0.59
0.5% s. hypochlorite	0.44	0.77
EH	0.33	0.58
EH + tannic acid	0.33	0.58
EH + b. detergent	0.22	0.19

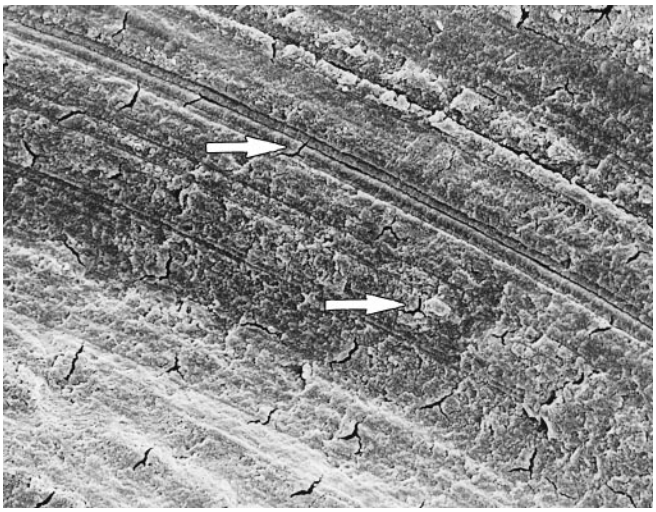

FIGURE 1 - Enamel hatchet associated with 5% tannic acid (mean score 0.89 ± 0.19). Electron micrograph showing the smear layer on a pulpal wall with a smooth appearance; arrows indicate some evidences of transversally cut dentinal tubules (2000 X).

FIGURE 2 - Biologic detergent (mean score 0.78 ± 0.17). Electron micrograph showing a lateral wall with an apparently thin smear layer, because some profiles of dentinal tubules (arrows) appear cut in longitudinal sections (400 X).

FIGURE 3 - 5% tannic acid (mean score 0.89 ± 0.17). Electron micrograph of a gingival wall showing the enamel with some removal of the smear layer, revealing the enamel prismatic structure (400 X).

As expected, the performance of phosphoric acid exceeded that of the others, completely removing the smear layer. The application methods were carried out based on the literature: cotton pellets are the instruments more frequently employed to take the substances into the cavities; acids, in general, were applied passively without rubbing, and organic solvents or detergents, with rubbing[2,3,9,21]. Moreover, the study of the treatments on MOD cavities allowed to observe their effects on different cavity walls, which would not be possible if just one surface of dental tissue had been employed. The enamel hatchet was used alone because it is difficult to apply this instrument with uniformity on all of the walls during a shorter period of time. Meanwhile, in association with the chemical substances, the period of application was the same (15 s). We attempted to use clinical situations adapted to experimental conditions. The statistical analyses compared each one of the treatments to each other.

The treatment of cavity walls with phosphoric acid has been applied in different concentrations for the removal of the smear layer, specially in adhesive restorative procedures. As the hazardous effects of phosphoric acid on dentin were demonstrated both in dentinal structure and in dentinal permeability, its use in small concentration for a short time has been suggested to minimize these effects and achieve the adhesion reactions[1,5,8,9,18,20].

This study demonstrated diverse performances of non or slightly demineralizing agents, according to the cavity walls in dentin. We have to consider the possible effect of mechanical procedures changing the initial result of chemical treatments. Thus, the act of scrubing with cotton pellets, or the use of enamel hatchet may be considered responsible for the final results on cavity walls. The simple friction of cotton pellets with water may result in some removal of the smear layer[5,9]. When phosphoric acid was associated with enamel hatchet, the smear layer was removed but some occluded tubules were detected. This fact may be explained by the greater compaction of the smear layer particles on the aperture of the tubules, which occurs during manual instrumentation[17].

The effect of a given treatment was diverse according to the kind of cavity wall. When tannic acid was applied alone, similar results on lateral and pulpal dentinal walls were observed. However, when tannic acid was associated with enamel hatchet, better results were observed on pulpal walls. The use of the enamel hatchet may be easier on pulpal walls and it may increase the effect of the chemical treatment. On the other hand, the use of biologic detergent applied with cotton pellets with rubbing produced better results on the dentin of lateral walls. The rubbing of cotton pellets might have been more effective on lateral walls.

We consider that treatment of the smear layer of enamel margins on gingival walls is important to prevent microleakage. Actually, we could not observe a uniform effect produced by any of the treatments on the enamel of gingival walls. These findings can justify the greater standard deviation of the statistical analysis, except for the tannic acid and biologic detergent associated with enamel hatchet, which produced the highest and the smallest mean score value, respectively. Examining the removal of the smear layer on the enamel surfaces of gingival walls, tannic acid had the higher mean score value among the treatments. This may be explained by its acidic nature[21] which imputes to it a slight demineralizing effect. In addition, the composition of the enamel smear layer is different from that of dentin, as the former tissue has greater mineral concentration.

Air/water spray could remove some smear particles, as shown by the use of the high speed handpiece with water cooling during cavity preparation[19,25]. Air/water spray treatment came in second place among the treatments on the lateral and gingival walls, although there was no statisti-

cally significant difference between this treatment and the others.

CONCLUSIONS

1. Phosphoric acid (37%) received the highest score due to complete removal of the smear layer;
2. non or slightly demineralizing agents presented diverse performances according to the cavity walls in dentin;
3. enamel hatchet with 5% tannic acid was more efficient on pulpal walls, while the biologic detergent was more efficient on lateral walls;
4. non or slightly demineralizing agents presented equivalent effect on the enamel of gingival walls.

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Um estudo comparativo cego de tratamentos químicos e/ou mecânicos da camada de esfregaço, de acordo com imagens da microscopia eletrônica de varredura, foi realizado. Analisou-se o efeito dos tratamentos sobre a camada de esfregaço de paredes de cavidades mésio-ocluso-distais, preparadas *in vitro*, em terceiros molares humanos. Os agentes empregados foram spray ar/água, ácido fosfórico a 37%, ácido tânico a 5%, detergente biológico, hipoclorito de sódio a 0,5% e machado para esmalte isoladamente ou associado a estas substâncias. Eletromicrografias foram avaliadas por três profissionais de acordo com o grau de visualização da dentina ou esmalte subjacentes. O ácido fosfórico recebeu as maiores graduações devido à remoção completa da camada de esfregaço. Análises estatísticas revelaram performances diversas entre tratamentos não/ou levemente desmineralizantes de acordo com as paredes cavitárias, em dentina e efeitos equivalentes entre si, em paredes de esmalte.

UNITERMOS: Camada de esfregaço; Dentina; Microscopia eletrônica de varredura.

BIBLIOGRAPHIC REFERENCES

1. ARAÚJO, M. A. J.; RODE, S. M.; VILLELA, L. C. *et al.* Avaliação qualitativa do efeito de agentes de limpeza na camada de lama dentinária: estudo ultra-estrutural em microscopia eletrônica de varredura. **Rev Odontol Univ São Paulo**, v. 12, n. 2, abr/jun, 1998.
2. BITTER, N. C. The effect of 25% tannic acid on prepared dentin: a scanning electron microscope methylene blue dye study. **J Prosthet Dent**, v. 64, n. 1, p. 12-16, July 1990.
3. BITTER, N. C. Tannic acid for smear removal: pilot study with scanning electron microscope. **J Prosthet Dent**, v. 61, n. 4, p. 503-507, Apr. 1989.
4. BRÄNNSTRÖM, M. **Dentin and pulp in restorative dentistry**. London : Wolfe, 1982. 125 p.
5. BRÄNNSTRÖM, M.; JOHNSON, G. Effect of various conditioners and cleaning agents on prepared dentin surfaces: a scanning electron microscopic investigation. **J Prosthet Dent**, v. 31, n. 4, p. 422-430, Apr. 1974.
6. BRÄNNSTRÖM, M.; GLANTZ, P. O.; NORDENVALL, K. J. The effect of some cleaning solutions on the morphology of dentin prepared in different ways: an *in vivo* study. **J Dent Child**, v. 46, n. 3, p. 19-23, July/Aug. 1979.
7. BROWN Jr, B. W.; HOLLANDER, M. **Statistics**: a biomedical introduction. New York : John Wiley & Sons, 1977. 456 p.
8. CHARLTON, D. G. Dentin bonding: past and present. **General Dent**, v. 44, n. 6, p. 498-507, Nov./Dec. 1996.
9. FRANCISCHONE, C. E.; CANDIDO, M. S. M.; BERBERT; A. *et al.* Efeito de alguns agentes de limpeza sobre a dentina, observado através da microscopia eletrônica de varredura. **Estomatol Cult (Bauru)**, v. 14, n. 1/2, p. 49-56, jan./dez. 1984.
10. JODAIKIN, A.; AUSTIN, J.C. The effects of cavity smear layer removal on experimental marginal leakage around amalgam restorations. **J Dent Res**, v. 60, n. 11, p. 1861-1866, Nov. 1981.
11. KAQUELER, J. C.; DECOMBAS, M.; LE MAY, O. **Structures bucco-dentaires en microscopie électronique a balayage**. Paris : Masson, 1989. 204 p.
12. NAKABAYASHI, N. Bonding of restorative materials to dentine: the present status in Japan. **Int Dent J**, v. 35, n. 2, p. 145-154, 1985.
13. NAKABAYASHI, N.; SAIMI, Y. Bonding to intact dentin. **J Dent Res**, v. 75, n. 9, p. 1706-1715, Sept. 1996.
14. NETER, J.; WASSERMAN, W.; KUTNER, M. H. **Applied linear statistical methods**. 3. ed. Homewwood: Richard D. Irwin, 1990. 1127 p.
15. PASHLEY, D. H.; CARVALHO, R. M. Dentine permeability and dentine adhesion. **J Dent**, v. 25, n. 5, p. 355-372, Sept. 1997.
16. PASHLEY, D. H.; DEPEW, D. D. Effects of the smear layer, copalite and oxalate on microleakage. **Operat Dent**, v. 11, n. 3, p. 95-102, 1986.
17. PASHLEY, D. H.; LEIBACH, J. G.; HORNER, J. A. The effects of burnishing NaF/Kaolin/Glycerin paste on dentin permeability. **J Periodontol**, v. 58, n. 1, p. 19-23, Jan. 1987.
18. PERDIGÃO, J.; LAMBRECHTS, P.; VAN MEERBEEK, B.; VANHERLE, G.; LOPES, A. L. B. Adesão aos tecidos dentários: o estado da arte II. **Rev Port Estomatol Cir Maxilofac**, v. 35, n. 4, p. 141-150, 1994.
19. SHORTALL, A.C. Cavity cleansers in restorative dentistry. Preliminary results from an in vitro scanning electron

LUZ, M. A. A. de C.; GARONE NETTO, N.; ARANA-CHAVEZ, V. E.; SOBRAL, M. A. P.; SINGER, J. da M. Evaluation of chemical and/or mechanical treatments of the smear layer as revealed by scanning electron microscopy – a blind comparative study. **Pesq Odont Bras**, v. 14, n. 2, p. 101-106, abr./jun. 2000.

microscope study. **Brit Dent J**, v. 150, n. 9, p. 243-247, May 1981.

20. SWIFT, E. J.; PERDIGÃO, J.; HEYMANN, H. O. Bonding to enamel and dentin: a brief history and state of the art, 1995. **Quintessence Int**, v. 26, n. 2, p. 95-110, Feb. 1995.
21. TAKAHASHI, H.; OKAMOTO, Y.; FUJINAKA, S. *et al.* Pilot study of exposure of the smear layer to tannic acid solutions. **J Prosth Dent**, v. 70, n. 3, p. 261-263, Sept. 1993.
22. VAN MEERBEEK, B.; PERDIGÃO, J.; LAMBRECHTS, P. *et al.* The clinical performance of adhesives. **J Dent**, v. 26, n. 1, p. 1-20, Jan. 1998.
23. VAN MEERBEEK, B.; DHEM, A.; GORET-NICAISE, M. *et al.* Comparative SEM and TEM examination of ultrastructure of resin-dentin interdifusion zone. **J Dent Res**, v. 72, n. 2, p. 495-501, Feb. 1993.
24. WHITE, G. J.; BEECH, D. R.; TYAS, M. J. Dentin smear layer: an asset or a liability for bonding? **Dent Mater**, v. 5, n. 6, p. 379-383, Nov. 1989.
25. ZAIMOGLU, A.; AYDIN, K. A. An evaluation of smear layer with various desensitizing agents after tooth preparation. **J Prosth Dent**, v. 68, n. 3, p. 450-457, Sept. 1990.

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Errata

Effect of professional dental prophylaxis with sodium bicarbonate jet on the cariogenic microbiota

Efeito da profilaxia profissional com jato de bicarbonato de sódio sobre a microbiota cariogênica

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O artigo "Effect of professional dental prophylaxis with sodium bicarbonate jet on the cariogenic microbiota", de Célia Regina Moreira LANZA, José Eduardo de Oliveira LIMA, Sergio Aparecido TORRES e Maria Aparecida de Andrade Moreira MACHADO, foi equivocadamente publicado como "artigo de divulgação" na edição de jan./mar. de 2000 da **Pesquisa Odontológica Brasileira** (v. 14, n. 1, p. 87-92); sua classificação correta é "artigo original".