Chemical Versus Conventional Caries Removal Techniques in Primary Teeth: A Microhardness Study

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The aim of this in vitro study was to assess the remaining dentinal surface after carious tooth tissue removal with a low speed conventional bur and two chemomechanical methods, (PapacárieTM and Carisolv®), using the microhardness test. It was concluded that the hardness of the remaining dentin after carious tissue removal was lower than that obtained on healthy dentin, without significance between the different means of carious tissue removal (p<0.05). **Key words:** carisolv, papacarie, primary teeth, tooth microhardness, caries J Clin Pediatr Dent 31(3):189-194, 2007

INTRODUCTION

Ithough there has been a substantial reduction of the prevalence of caries in industrialized countries, this disease continues to be widespread in the world. Once it has become installed, it is of fundamental importance to use conservative procedures that simultaneously prevent lesion progress and minimize healthy tooth structure wear. Ideally, the methods used to remove carious tissue should be capable of distinguishing the internal carious tissue layer from the more superficial and highly infected tissue, in which collagen fibers can no longer be remineralized.^{12.3} In addition, these methods must be comfortable for the patient, easy to use, noiseless, painless and must not cause vibration.

The following are among the main disadvantages of the traditional method using a rotary instrument: the possibility of overextending the cavity, healthy tissue removal, pressure and heat on the pulp, vibration, noise, pain stimulus and the need of local anesthetic, a procedure that causes aversion in many patients, specially children. New methods of carious tissue removal have been developed as an alternative to traditional treatment, among which one may mention laser, air abrasion, ultrasound, and chemomechanical removal.

The objective of chemomechanical substances is to remove the most external portion (infected layer), leaving the affected demineralized dentin that is capable of being remineralized and repaired.⁵ Chemomechanical methods are said to remove only the infected dentin where collagen is degraded, maintaining the demineralized

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portion.

Innumerable studies have been conducted to assess the efficacy and clinical safety of Carisolv®, many of which have pointed out that the majority of patients felt no discomfort during treatment,^{5,6,7} it was hardly ever necessary to use local anesthesia ^{8,9} and carious tissue removal was efficient.^{10,11} As a disadvantage, this method was less efficient in comparison with the traditional method for carious tissue removal, making it necessary to spend more clinical time.^{47,11,12,13} In addition, the high selling price to the consumer was an obstacle to the regular use of the method in clinical routine.

With the intention of presenting a chemomechanical caries removal product that cost less than Carisolv®, in 2003 Papacárie®, a material composed of papain, chloramin and toluidine blue, was launched. Papain is an endoprotein, with bacteriostatic, bactericide and anti-inflammatory activity.⁵ Chloramin, a compound that contains chlorine and ammonia, presents bactericide and disinfectant properties, is used to irrigate root canals and to chemically soften carious dentin, so that the degraded portion of the carious dentin collagen is chlorinated by the solution used for chemical and mechanical caries removal.⁹

Thus, the purpose of this study was to compare the Knoop microhardness of sound dentin before and after carious tissue removal using the two chemical-mechanical methods, and the conventional method.

MATERIAL AND METHODS

This research protocol received previous consent from the Research Ethics Committee, School of Dentistry, University of São Paulo (protocol number 167/04).

Thirty extracted central primary incisors, with active carious cavities on one proximal surface, were divided into three experimental groups as follows, in accordance with the carious tissue removal method: conventional mechanical treatment – slow speed rotary instrument – and two chemomechanical methods - PapacárieTM and Carisolv®.

Carious tissue removal using the conventional technique was performed with a spherical steel bur (Wilcos do Brasil, Petrópolis-Brazil) with the largest diameter compatible with the cavity size, at

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low speed, under water cooling, by a single operator. In order to gauge carious tissue removal, a dental explorer was used to check, until hard dentin was obtained.

For the PapacárieTM (Fórmula & Ação, São Paulo, Brazil) and Carisolv® (Medi Team, Gothemburg, Sweden) groups, the product was applied and left in the cavity for 30 seconds, and carious dentin was afterwards removed with a blunt Maileffer curette (Bailagues, Switzerland) that comes with the Carisolv® system kit. The gel was reapplied until it presented a light coloring, indicative of non-existence of softened carious tissue, and confirmed with the use of the dental explorer, to assess the remaining dentin hardness.

Preparing test specimens for microhardness test

After carious tissue removal, the teeth were longitudinally sectioned - under cooling - in the mesio distal direction of the crown, using a precision cutting machine (Labcut 1010 - Extec Corp. Enfield, USA), at the center of the cavity, until two sections were obtained. One of the sections was embedded in epoxy resin (Resigel - Redefibra de São Paulo), so that the area to be analyzed remained exposed. Polishing was done in a rotary polisher (Ecomet 4, Buehler, Lake Bluff, USA), with 600, 1000, 1200, 2000 and 4000 grit abrasive paper, and final polishing was done with a felt disk and diamond paste of 1µm and 1/4µm (Arotec, São Paulo, Brazil). The 600 grit abrasive paper was used for a period of 30 seconds, and the remainder for 60 seconds under water cooling, and the polishing obtained was checked before going on to a finer grit. This preparation was considered ideal when, under optic microscopy, the specimens were shown to be shiny and without presence of scratches. At the last stage of polishing, the test specimens were placed in ultrasound (Thoron) for 12 minutes, to remove eventual residues.

The microhardness test was performed on sound dentin and on healthy dentin of the same specimen. For this analysis, a microhardness meter Shimadzu HMV II (Kyoto, Japan) was used, with a Knoop indenter using a static load of 25 grams applied for 30 seconds on the sound dentin and 10 grams for 30 seconds on treated dentin. On the dentin submitted to carious tissue removal, 21 indentations were made - three at each distance – starting from 50, 100, 150, 200, 300, 400 and 500µm from the base of the carious cavity, and on the sound dentin 24 indentations were made – three at each distance – from 100, 150, 200, 300, 400, 500, 1000, 1500µm from the amelodentinal junction. The indentations were made with a distance of 100µm between them.



Figure 1: Points and intervals of microhardness assessment, in dentin submitted to caries removal at a location where dentin was submitted to caries removal.



Figure 2: Points and intervals of microhardness assessment, in sound dentin.

RESULTS

The factors analyzed in this study were: "type of treatment" (rotatory instrument and chemomechanical methods), "indentation intervals", and "type of tissue" (sound and carious).

Results of microhardness values were presented separately because the indentations were made at different points on each side of the tooth (from the cavity's pulpal wall on treated dentin and from the amelodentinal junction on sound dentin).

Regarding treated dentin, the microhardness values obtained for the different types of treatment (rotary instrument and chemomechanical methods) did not show statistically significant differences (p>0.05). Similarly, values obtained at the assessment intervals were not significantly different either (p>0.05). This indicates that microhardness values were similar for the different treatments and for the different intervals used. However, the interaction between "treatment" and "intervals" was significant, showing that the variation in microhardness was different for the different intervals used in respect to the treatment group (Table 1).

Table 1 – Mean Knoop microhardness (kgf/mm²) of dentin after carious tissue removal

Intervals	Bur	Papacárie®	Carisolv®
50 _µm	8.17±2.60	8.47±3.29	6.77±2.08
100 µm	7.00±2.36	8.53±2.38	7.33±2.00
150 µm	6.84±1.44	8.53±2.13	8.19±2.30
200 µm	6.79±2.02	8.50±1.74	9.06±2.51
300 µm	6.50±1.78	8.65±1.63	9.21±2.58
400 µm	6.72±1.76	8.96±2.26	9.00±3.29
500 µm	6.23±1.05	9.04±2.51	9.87±5.22

On Table 1, it is possible to observe that the rotatory instrument group (bur) showed a small reduction in microhardness value as the depth increased, while the PapacárieTM group was homogeneous at all intervals. In the Carisolv® group a lower microhardness value is observed immediately below the cavity floor, increasing at the deeper intervals. Yet, for this group, the difference was only significant between the intervals of 50 and 500 μ m.

On sound dentin, the microhardness values were similar for all

Table 2 – Mean Knoop microhardness	(kgf/mm ²) of dentin on the sound side
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Intervals	Means	
100 µm	15.53± 6.57	
150 µm	14.89± 7.16	
200 µm	14.34± 7.99	
300 µm	13.45± 8.62	
400 µm	12.77± 7.91	
500 µm	12.35± 7.39	
1000 µm	8.17± 3.22	
1500 µm	7.48± 2.30	

Table 3 – Mean Knoop microhardness values (kgf/mm²) of dentin on the carious and sound sides

Side	Means		
Carious	7.97±1.02	p< 0.05	
Healthy	12.40±3.21		

 Table 4 Mean Knoop microhardness values (kgf/mm²) of dentin on the carious and sound sides

Side	Means	
Carious	7.81±2.72	p< 0.05
Healthy	9.47±3.90	

treatment groups and were only statistically different for the "Interval" (p<0.05) variable.

The mean microhardness values for the treatment groups, as well as the Tukey contrast value are presented on Table 2.

There was no difference between the microhardness values at the first intervals (100, 150, 200, 300, 400 μ m) but a statistically significant difference was obtained between the intervals of 100 μ m and 500 μ m, as well as between intervals of 500, 1000 and 1500 μ m. As expected, on the sound side of the tooth, no factor influenced the dentinal hardness as there were no differences between the treatment groups or for the interaction "treatment" and "interval"; thus confirming that all of the teeth used in this study presented with a similar hardness characteristic.

Afterwards, a comparison was made between the microhardness values of the different types of dental tissue (sound side and carious side after caries removal), and this analysis was done in two ways. In the first assessment, the mean values of each tooth on both sides were considered. No statistical difference was observed between the different treatment methods (p>0.05) but there was a difference between the sound side and the side submitted to carious tissue removal, with the latter presenting a lower value (p<0.05).

For the second comparison, microhardness hardness value of the dentin treated with different methods was considered, at the point of 50µm from the cavity, that is, the measurement obtained closest to the cavity floor. The microhardness at the equivalent point on the sound side was calculated, using a regression curve with the best fit. As in the previous analysis, a statistically significant difference was

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found between the sound side and the carious side (p < 0.05). The sound and carious sides' means are presented on Table 4.

DISCUSSION

Caries is a disease that presents high incidence from the earliest ages and promotes tooth structure loss, harming the individual's oral and general health. Generally speaking, when the dentin is compromised and it is difficult to control biofilm formation on the lesion, it is necessary to remove the tissue involved to control the development of the disease. In addition, removal of the softened dentin, or part of it, is a basic condition for supporting the future restoration.

Although the conventional carious tissue removal method, with the use of high and low speed burs, allows fast treatment, its cut may promote unnecessary structure removal, with consequent weakening of the tooth remainder, as well as pulp injuries.

As a result of the above-mentioned aspects, the use of chemomechanical carious tissue removal has grown considerably, not only because of the advantages previously pointed out,^{5,6,7,18} but also because some authors have mentioned that these techniques are capable of removing only the infected, necrotic dentin, incapable of being remineralized, thus guaranteeing the preservation of the lower, non-infected layer.

The clinical methods for differentiating between these two layers are subjective, and in the majority of cases, dentists find it difficult to identify the ideal moment to stop removing dentinal tissue.

In this study, mechanical carious tissue removal was performed until sound dentin was obtained, gauged by the test performed with the exploratory probe.^{11,19,20,21} In chemomechanical removal, the manufacturers' instructions were adopted, and caries removal ceased when the gel attained a clear (non-turbid) appearance. Although the PapacárieTM manufacturer recommends the use of old, blunt curettes, it was opted to use those supplied with the Carisolv[®] Kit in order to obtain standardization.

Microhardness analysis has been used as a method to assess loss and reincorporation of minerals to the dental tissue, because the reduction in the numerical hardness value presents a linear relation to mineral loss.^{16,17} Especially Knoop hardness, since it represents a significant correlation with the amount of mineral loss from the tooth structure.^{16,17}

Literature clearly presents the microhardness values of permanent teeth, but this is not the case for primary teeth. In spite of the differences existing between primary and permanent teeth as far as the degree of mineralization, structure, mineral loss and reactivity to fluoride are concerned^{22,23} there are few studies that specifically deal with primary tooth microhardness. This scarcity of investigations makes it difficult to compare studies that use this assessment methodology, since only one study pointed out that the transversal microhardness values of dentin are lower in primary than in permanent teeth.²⁴

There appears to be an agreement among the majority of authors regarding the fact that the carious dentin microhardness values and that of dentin remaining after carious tissue removal are lower than those of sound dentin.^{2,11,25,26,27} The microhardness values (KHN) of sound dentin and carious dentin – or after its removal - obtained in the above-mentioned studies range from 30 to 70 and 4.3 to 26.4 respectively, from which one is able to notice a great variability between the 2 types of dentin. When dentin is affected by the carious process, its structure is altered, the dentinal tubules become

occluded with mineral content and their hardness diminishes in comparison to that of sound dentin.^{26,28} Thus, since two types of tissues with different structures were concerned, different loads were used because it would not be possible to obtain indentations of a similar quality otherwise. The Knoop indenter loads used were 10 grams for 30 seconds on carious dentin, as recommended by some authors,^{29,30,31,32} and 25 grams for 30 seconds on sound dentin, such loads are considered to be sufficient to permanently deform the assessed structures, without the occurrence of elastic deformations that could lead to any alteration.³³ These loads resulted in defined indentations with minimized fractures around them.

The influence of load variation on the microhardness values is also discussed in the literature. There are reports that variations in microhardness occur when different loads are used,^{34,35} and that increase in load involves the increase in enamel microhardness and reduction in dentin microhardness.³⁶ Contrary to these observations is the report that the variation occurs in the size of the indentation, theoretically not affecting the final Knoop microhardness result.³⁷

This work was done with only one group of teeth (central incisors) in an endeavor to homogenize the sample. Some authors affirmed that there is no clear trend of variation in microhardness between the different types of permanent teeth.³⁴ It was demonstrated, however, that primary molar enamel presents greater hardness than canines and incisors,³⁸ and the same could occur with dentin. In the present study, incisors were also used because they were easier to obtain, since the molars had a large number of lesions with pulp chamber exposure.

After carious tissue removal, dentin microhardness did not differ between the treatment groups and at the different distances, and were equivalent to a study that showed similar microhardness values after mechanical and chemomechanical caries removal.¹¹ Thus, from the carious dentin tissue removal point of view, there was similarity between the two methods – mechanical and chemomechanical, and even between Carisolv® and PapacárieTM. On the other hand, there was a report that stated that the microhardness value of dentin submitted to mechanical removal was statistically higher than that of dentin submitted to chemomechanical removal (Carisolv®).³⁰

The parameter most used for the carious tissue removal procedure is the clinical one, in which the dentin hardness is assessed.³⁹ However, this method is not considered to be precise because it is empirical, it does not generate a safe diagnosis, and it does not provide accurate information about the amount of tissue to be removed. On the other hand, it is believed that lesion hardness determination is an acceptable parameter for assessing demineralization of the affected dentin, as one study found a lower number of cariogenic bacteria in harder dentin than in more softened dentin.⁴⁰

It is interesting to find that, in the group treated with conventional bur, the values were similar at all depths and that the same occurred for the PapacárieTM group. Whereas in the Carisolv® group, the initial values were lower for the first indentations, slowly rising as a result of increase in interval, although it was only between the first and the last indentation that the difference was significant. These findings may be associated with the composition of the product, as it is reported that hypochlorite causes greater dentin softening, and was aggressive even in healthy tissues.^{32,41} Even though these initial values were lower, they did not differ statistically from the other two methods.

The results found suggest that the two chemomechanical caries

removal methods remove not only the infected dentin layer, but also act on the affected dentin layer, removing it completely or partially, in this case resulting in a very thin layer, less than 50µm, since it was at this distance that the first indentation was made. It is postulated that the interaction between the chloride and collagen does not occur in the mineralized tissue and protects the collagen fibers from the action of sodium hypochlorite, and is a selective and self-limiting technique, with specificity for carious tissue.^{59,42} Other investigations have shown that the use of Carisolv® removes part of the affected dentin and does not preserve the dentinal collagen¹⁹; and there is no difference of microhardness between cavities treated with Carisolv® and sound dentin, as the amounts of calcium and phosphate remaining in the two tissues are similar.¹⁰

The sound dentin microhardness values were higher closer to the amelodentinal regions, and decreased in the direction of the pulp chamber, being statistically lower at the intervals of 1000 and 1500_m from the amelodentinal junction, when compared with the values of the initial distances. These results are in agreement with the findings of various authors,^{26,29,30,39} although some mention lower hardness in the amelodentinal junction region.^{25,34}

The reduction in microhardness could be associated with the distribution of the dentinal tubules, which are presented continuously between the enamel and the pulp, and range in density from 15.000/mm2 – at the enamel and dentin interface - to 65.000/mm2 – close to the pulp chamber.⁴³ Furthermore, it is believed that the reduction in microhardness observed in the proximity of the pulp is a result of the increased volume of the tubules and possible changes in mineral density.⁴⁴

Microhardness values of both dentin submitted to carious tissue removal and sound dentin were lower than the values found in literature. Such results may have occurred due to the low concentration of calcium and phosphate in teeth used⁴⁵ and because central incisors enamel, which present lower microhardness values than canines and molars in enamel were used.³⁸ Also, another explanation may be that the teeth were obtained from a bank were origin, age and storage time were not registered.

A comparison between the microhardness values at the same distances, on both the sound side and the side submitted to some type of treatment becomes difficult, due to tissue loss caused by the carious process, the first indentation on the treated side would be closer to the pulp, differently from that on the healthy side, which would be at the amelodentinal junction. The majority of studies correlate these values, but certainly do not do so at the same distance.^{25,10}

To compare the microhardness values between the tooth tissue remaining after infected tissue removal and the sound tissue, two types of analyses were performed: in the first analysis, the means of the teeth on the side submitted to the treatments and those obtained on the sound side were compared; in another analysis, the microhardness value of treated dentin, at 50µm from the base of the carious cavity was compared with the estimated value on the sound side at the same distance, obtained by regression analysis. Both analyses showed statistically significant differences between the two sides, with greater microhardness on the sound side, which corroborates the findings in the literature.^{25,26,27} The microhardness values obtained after caries removal were compared with the value found at the distance of 1000µm below the cavity, and no difference was found between the two measurements.¹⁰ In the present investigation it was opted not to make this comparison, since the microhardness value at

the distance of 1000µm on the healthy side was significantly lower than those at the initial distances, and also because there were differences in the distances from the cavities.

Many authors mention that the chemomechanical method removes only the infected dentin, leaving the affected dentin that would still be softened. In this study, the results showed that the hardness of the remaining dentin was similar to that obtained with the conventional burr removal method, probably removing all the softened tissue. Obviously, with any method, the moment to stop removing carious tissue is determined by the professional, but when the manufacturer's instructions – to remove the tissue while the gel is turbid – were respected, the removal of all compromised structure was observed.

CONCLUSIONS

In accordance with the results obtained in this study, it may be concluded that:

1. The microhardness of the dentin remaining after removal with a rotary cutting instrument and chemomechanical removal (Carisolv® and Papacárie®) was similar.

2. Sound dentin has a higher microhardness value than the dentin that remains after being submitted to carious tissue removal.

REFERENCES

- Beeley JA, Yip HK & Stevenson AG. Chemomechanical caries removal: a review of the techniques and latest developments. British Dental Journal 188(8):427-430, 2000
- 2. Fusayama T. Two layers of carious dentin; diagnosis and treatment Operative Dentistry 22(3): 401-411, 1979
- Ogushi K "& Fusayama T. Electron microscopic structure of the two layers of carious dentine Journal of Dental Research54(5):1019-1026, 1975
- Banerjee A, Kidd EAM & Watson TF. In vitro evaluation of five alter native methods of carious dentine excavation. Caries Research 34(2):144-150, 2000
- Bussadori SK, Castro LC, Galvao AC. Papain gel: a new chemomechanical caries removal agent. J Clin Pediatr Dent: 30(2):115-9, 2005
- Munshi AK, Hedge AM & Shetty PK. Clinical evaluation of Carisolv in the chemico-mechanical removal of carious dentin Journal Clinical of Pediatric Dentistry 26(1): 49-54, 2001
- Nadanovsky P, Carneiro FC & Mello FS. Removal of carious using only hand instruments: a comparison of mechanical and chemomechanical methods Caries Research 35(5):384-389, 2001
- Kakaboura A, Masouras C, Staikou O & Vougiouklakis G. A comparative clinical study on the Carisolv caries removal method Quintessence International 34(4):269-271, 2003
- Maragakis GM, Hahn P& Hellwing E. Clinical evaluation of chemomechanical caries removal in primary molars and its acceptance by patients Caries Research: 35(3):205-210, 2001.
- Hossain M, Nakamura Y, Tamaki Y, Yamada Y, Jayawardena JA & Matsumoto K. Dentinal composition and Knoop hardness measurements of cavity floor following carious dentin removal with Carisolv Operative Dentistry: 28(4): 346-351, 2003
- Fluckiger L, Waltimo T, Stich H & Lussi A. Comparison of chemomechanical caries removal using Carisolv or conventional hand excavation in deciduous teeth in vitro Journal of Dentistry 33(2): 87-90, 2005
- Fure S, Lingstrom P & Birkhed D. Evaluation of Carisolv for the chemomechanical removal of primary root caries in vivo Caries Research 34(3):275-280, 2000.
- Yazici AR, Atilia P, Ozgunaltay G & Muftuoglu S. In vitro comparison of the efficacy of Carisolv and conventional rotary instrumental in caries removal Journal of Oral Rehabilitation 30(12):1177-1182, 2003

- 14. Chaussain-Miller C, Decup F, Domejean-Oliaguet S, Gillet D, Guigand M & Kaleka R. Clinical evaluation of the Carisolv chemomechanical caries removal technique according to the site stage concept, a revised caries classification system Clinical Oral Investigation 7(1) 32-37, 2003
- Candido LC (2004) Web site evolution; Retrivied online November 23, 2004 from: http://www.feridologo.com.br/curpapaina.htm
- 16. Feagin F, Patel PR, Koulourides T & Pigman W. Study of the effect of calcium, phosphate, fluoride and hydrogen ion concentrations on the remineralization of partially demineralized human and bovine enamel surfaces Archieves of Oral Biology 16(5) 535-548, 1971
- Koulorides T & Housch T. Hardness testing and microradiography of enamel in relation to intraoral de-and remineralization. In: Leach AS & Edgar WM. Demineralization and remineralization of the teeth. Oxford: IRL Press 255-72, 1983
- Rafique S, Fiske J & Banerjee A. Clinical trial of an air-abrasion chemo mechanical operative procedure for the restorative treatment of dental patients Caries Research 37(5): 360-364, 2003
- Cederlund A, Seven L & Blomlof J. Effect of a chemomechanical caries removal system (Carisolv) on dentin topography of non-carious dentin Acta Odontologica Scandinavica 57(4):185-189, 1999
- Haak R, Wicht MJ & Noack MJ. Does chemomechanical caries removal affects dentine adhesion? European Journal of Oral Science 108(5): 449-455, 2000
- Splieth C, Rosin M & Gellisen B. Determination of residual dentine caries after conventional mechanical and chemomechanical caries removal with Carisolv Clinical Oral Investigation 5(4): 250-253, 2001
- Fejerskov O. Concepts on dental caries and their consequences for understanding the disease Community Dentistry and Oral Epidemiology 25(1): 5-12, 1997
- 23. Sonju Clasen AB, Ofaard B, Duschner H, Ruben J, Arends J & Sonju T. Caries development in fluoridated and non-fluoridated deciduous and permanent enamel in situ examined by microradiography and confocal laser scanning microscopy Advances in Dental Research 11(4): 442-447, 1997
- Johnsen DC, Schechner TG & Gerstenmaiter JH. Proportional changes in caries patterns from early to primary dentition Journal of Public Health 47(1): 5-9, 1987
- Banerjee A, Sheriff M, Kidd EA & Watson TF. A confocal microscopic study relating the autofluorescence of carious dentine to its micro hardness British Dental Journal 187(4): 206-210, 1999
- Hosoya Y, Marshall SJ, Watanabe LG & Marshall GW. Microhardness of carious deciduous dentin Operative Dentistry 25(2): 81-89, 2000
- Iost HI, Costa JH, Rodrigues HH, Rocca RA. Dureza e contaminação bacteriana da dentina após remoção da lesão de cárie Revista ABO Nacional 3(1): 25-29, 1995
- Marshall GW Jr, Marshall SJ, Kinney JH & Balooch M. The dentin substrate: structure and properties related to bonding Journal of Dentistry 25(6): 441-458, 1997
- Craig RG, Gehring PE & Peyton FA. Relation of structure to the micro hardness of human dentin Journal of Dental Research 38(3): 624-630, 1959
- Hosoya Y, Ono T, Grayson W & Marshall Jr. Microhardness of carious primary canine dentin Pediatric Dental Journal 12(1): 91-98, 2002.
- Santiago BM, Ventin DA, Primo LG & Barcelos R. Microhardness of dentin underlying ART restorations in primary molars: an in vivo pilot study British Dental Journal 2(23): 103-106, 2005.
- Tonami K, Araki K, Mataki S & Kurosaki N. Effects of chloramines and sodium hypochlorite on carious dentin Journal of Medical Dental Science 50(2): 139-146, 2003
- Hara AT, Turssi CP, Serra MC & Nogueira MCS. Extent of the cariostatic effect on root dentin provided by fluoride containing restorative materials Operative Dentistry 27(5):480-7, 2002
- Craig RG & Peyton FA. The microhardenss of enamel and dentin Journal of Dental Research 37(4):661-668, 1958
- 35. Mahoney E, Holt A, Swain M & Kilpatrick N. The hardness and mod-

ulus of elasticity of primary molar teeth: an ultra-micro-indentation study Journal of Dentistry 28(8):589-594, 2000.

- Hegdahl T & Hagebo T . The load dependence in micro indentation hardness testing of enamel and dentin Scandinavia Journal of Dental Research 80: 449-452, 1972
- Zárater- Pereira P. Estudo in situ sobre a ação da própolis de Apis melifera no desenvolvimento da cárie dentária e na formação do biofilme dental [Doctorate Thesis]. São Paulo: Faculdade de Odontologia da USP; 2003.
- Rocha RO, Corrêa FNPC & Rodrigues CRMD.Microdureza superficial e transversal do esmalte de dentes decíduos Brazilian Oral Research 18 Abstract # PC148 p-221, 2004
- Fusayama T, Okuse K & Hosada. H Relationship between discoloration and microbial invasion in carious dentin Journal of Dentistry 45(4):1033-1046, 1966
- Kidd EAM, Joyston- Bechal S, Beighton D.The use of a caries detector dye during cavity preparation: a microbiological assessment British Dental Journal 174 (7):245-248, 1993
- Goldman M & Kronam JH. A preliminary report on a chemomechanical menas of removing caries Journal of American Dental Association 93(6):1149-1153, 1976
- 42. Dammaschke T, Stratmann U, Morkys K, Kaup M & Ott KHR. Reaction of sound and demineralized dentine to Carisolv *in vivo* and *in vitro* Journal of Dentistry 30(1):50-65, 2002.
- Fosse G, Saele PK & Eibe R. Numerical density and distributional pattern of dentine tubules Acta Odontologica Scandinavcia 50(7) 201-210, 1992
- Pashley D, Okabe A, Parham P. The relationship between dentin micro hardness and tubule density Endodontic Dental Traumatology 1(5):176-179, 1985
- 45. Lakomaa E & Rytomaa I. Mineral composition of enamel and dentin of primary and permanent teeth in Finland Scandinavica Journal of Dental Research 85(2):89-95, 1977