Dentin Rehardening after Indirect Pulp Treatment in Primary Teeth

R. Franzon, DDS, MS M. Gomes, DDS, MS C.M. Pitoni, DDS, MS, PhD
C.P. Bergmann, DDS, MS, PhD F.B. Araujo, DDS, MS, PhD

ABSTRACT

Purpose: The purpose of this study was to investigate dentin rehardening in the remaining carious dentin after indirect pulp treatment (IPT) using microhardness analysis after 37 to 71 months.

Methods: Eighteen teeth submitted to IPT and capped with calcium hydroxide (CH) or gutta-percha (GP) were evaluated (treated group). Ten sound molars and 10 molars with deep acute carious lesions were selected to serve as positive and negative control groups, respectively. In the treated group, restorations and pulp-capping materials were removed. In the positive control group, 3- to 4-mm deep cavities were prepared. In the negative control group, the carious tissue was removed. Microhardness analysis was performed at 10-, 35-, 60-, 85-, and 110-μm depths. Data were analyzed using 1-way analysis of variance ($P<.05$).

Results: Microhardness values for sound, carious, and treated groups at 10-, 35-, 60-, 85-, and 110-μm depths showed a statistically significant difference ($P\leq.01$) among the groups for microhardness. No difference was observed between CH- and GP-treated groups for microhardness.

Conclusion: The results showed a hardness increase in treated teeth when compared to carious teeth in all dentin depths investigated, suggesting mineral gain after treatment.
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Dental caries continues to be a highly prevalent disease among preschoolers.[1] Indirect pulp treatment (IPT) is indicated for deep carious lesions (excluding a chance of a pulp exposure) in which the nonremineralizable tissue is removed and a thin layer of caries is left in the deepest site of the cavity, avoiding the possibility of pulp exposure.[2] The superficial layer of the carious dentin (which contains the majority of micro-organisms) must be removed,[3,4] and the affected dentin or the decalcified dentin could be left at the deepest portions of the cavity preparation, considering that this area might be remineralized and has a small amount of micro-organisms. Under these circumstances, residual bacteria are isolated from nutrient sources, stop proliferating, and die.[5,6] Studies have shown a reduction in the number of micro-organisms and the arrestment of active lesions after IPT.[7-9]

There is evidence that IPT can be completed in a single clinical session,[4,9-12] considering the satisfactory clinical and radiographic findings of previous studies with primary teeth[4,7,9-11,13-16] that have supported

Drs. Franzon and Gomes are postgraduate students, Dr. Pitoni is postgraduate professor, and Dr. Araujo is associate professor, all in the Department of Pediatric Dentistry and Orthodontics, School of Dentistry, and Dr. Bergmann is associate professor in the Department of Materials, School of Engineering, all at Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul, Brazil. Correspond with Dr. de Araujo at fernando.araujo@ufrgs.br

the choice for a 1-visit treatment. A single clinical session eliminates the need to reopen the capped tooth within approximately 3 months after IPT and remove the remaining dentin. In addition, this procedure may be a definitive treatment for the primary dentition, since primary teeth have a defined biological cycle in the oral cavity.

The dentin microstructure in deciduous teeth has received limited attention. Studies have described dentin remineralization after IPT based on the increased content of calcium[4] and phosphorus.[17] A pilot study observed a dentin microhardness increase after alternative restorative treatment in vivo.[18]

The dental literature shows that dentin hardness decreases from the dentin enamel junction to the pulp chamber wall in deciduous[19] and permanent sound teeth.[20] Also, the area under carious tissue is the softer region of carious dentin. Hardness measurements cannot be performed for infected dentin since it is too soft.[19]

The purpose of this study was to investigate dentin rehardening in the remaining carious dentin after indirect pulp treatment using microhardness analysis after 37 to 71 months.

METHODS

The sample consisted of 42 primary maxillary and mandibular molars with deep carious lesions from 20 4- to 7-year-old patients. The main inclusion criteria were: absence of spontaneous pain, swelling, or fistula; and tooth mobility not compatible with chronological age and all margins in the enamel. Radiographic criteria for inclusion were teeth with caries involving at least half the dentin thickness, but no radiographic carious contact with the pulp—suggesting pulpal degeneration, such as internal resorption or furcation radiolucency.

Treatment procedures included[9]:

1. regional anesthesia and rubber dam isolation;
2. removal of decayed tissue in the lateral walls with low-speed round burs and spoon excavators;
3. removal of necrotic tissue located at the pulpal wall, with a smooth and fragmented appearance, with only spoon excavators;
4. cleaning of the cavity and clinically evaluating the tissue's color and hardness;
5. microbiologically analyzing samples of remaining decayed tissue, during which no pulp exposure occurred;
6. dividing the sample teeth into 2 groups according to the liner material on pulp wall:
    a. calcium hydroxide—remineralization inductive material (Hydro C, Caulk Dentsply, Petrópolis, Rio de Janeiro, Brazil); and
    b. gutta-percha—inert material (gutta-percha, Caulk Dentsply). The materials were placed to cover the remaining carious dentin;
7. restoring the cavities with a resin-based composite (Filtek Z250, 3M ESPE, St. Paul, Minn); and
8. radiographic examination (baseline).

After 4 to 7 months, the teeth were clinically and radiographically evaluated. The cavity was reopened, and the dentine was sampled for microbiological analysis. The teeth were then restored again with the same previous materials.[9]

After follow-up 36 months later, 29 teeth were re-evaluated. Twenty-three showed clinical and radiographic signs of normality characterized by absence of pain, fistula, swelling of periodontal tissues, tooth mobility not associated with root resorption, and periapical or furcation radiolucency.[12] Children were included in a maintenance program, and the teeth were collected after exfoliation.

TEST GROUP (TREATED)

Of the 18 collected teeth, 6 were capped with calcium "hydroxide (CH) and 12 with gutta-percha (GP). On average, treated teeth had clinical and radiographic success of 4 years and 5 months of follow-up (median=50 months). Success was characterized by the absence of pain, fistula, swelling of periodontal tissues, tooth mobility not associated with root resorption, and periapical or furcation radiolucency. All the teeth collected after exfoliation were included in the study.

NEGATIVE (CARIOUS) AND POSITIVE (SOUND) CONTROL GROUPS

The positive control group consisted of 10 sound primary maxillary and mandibular molars. The negative control group consisted of 10 primary maxillary and mandibular molars with deep, active, carious lesions (near the pulp) diagnosed clinically and radiographically. The teeth were collected until the established sample was completed.

All teeth (sound, carious, and treated) were obtained after natural exfoliation or extraction for reasons not related to this study and were stored in saline.

At the beginning of treatment, subjects presented an active disease profile and were enrolled in a dental care program that included routine professional monitoring of their oral health status. They received treatment for lesions arrestment, including extraction of unrestorable teeth, pulp treatment, and restoration of carious lesions, if necessary, to allow the subjects to be able to control the bacterial biofilm by themselves. In addition, topical fluoride was professionally applied at regular intervals according to the caries risk assessment. Children were maintained in the program until the last evaluation of the present study and were included in a maintenance program after study completion.

All children's parents/caregivers were told of the study's purposes and read and signed an informed consent form for donation of the exfoliated primary teeth.

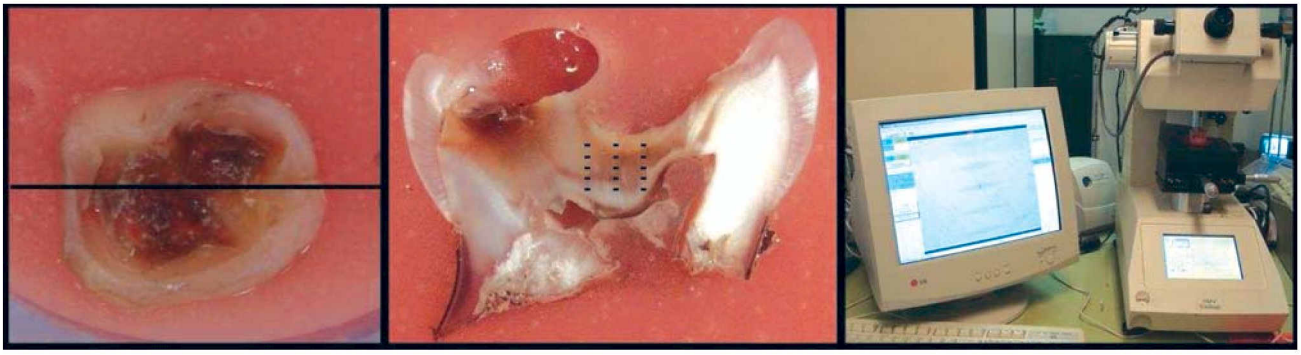

Figure 1. Specimens preparation and microhardness analysis.

Table 1. Mean (±SD) Microhardness Values (Knoop Number Hardness) of Calcium Hydroxide and Gutta-Percha in All Measured Depths*

Depth (mm)	Experimental group†	N	Mean (±SD)	*P*-value
10	CH	5	35.8±16.3	.77
	GP	10	39.5±23.7	
35	CH	5	32.8±14.3	.51
	GP	10	40.6±23.6	
60	CH	5	33.5±10.9	.49
	GP	10	41.9±25.1	
85	CH	5	35.7±9.9	.53
	GP	10	42.6±22.5	
110	CH	5	37.5±12.8	.54
	GP	10	44.3±22.1	

** No statistically significant difference existed among the groups at all evaluated depths.*

† CH=calcium hyroxide; GP=gutta-percha.

The study protocol was approved by the Ethics in Research Committee of the Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande du Sul, Brazil.

SAMPLE PREPARATION

In the treated group, restorations were removed from the teeth using spherical diamond burs no. 1016 (KG Sorensen, São Paulo, Brazil) at high speed under continuous air/water spray cooling up near the cavity floor. The capping materials, CH and GP, were carefully removed with an excavator, with no pressure on the remaining dentin.

In the positive control group (sound), cavities were prepared in dentin with spherical diamond burs no. 1016 (KG Sorensen) at high speed under continuous air/water spray cooling up to a depth of 3 to 4 mm, which was the average cavity depth observed in the treated group after removal of the restorations. In the negative control group (carious), the infected tissue was removed in vitro according to the treated group's same IPT criteria.[9]

MICROHARDNESS PREPARATION

The specimens in all groups were embedded in autopolymerizing acrylic resin (Clássico Dental Products, São Paulo). They were sectioned mesiodistally using a sectioning machine (Isomet 2000, Buehler, Lake Bluff, Ill) with a water-cooled 0.80-mm-thick diamond saw at low speed (3.500 rpm) and with a 250-g load.

The sections were included in acrylic resin and polished with water-cooled 1,200-grit silicon carbine paper for 5 minutes in a polishing machine (Strues, Copenhagen, Denmark) with a load of 100 N and a speed of 150 rpm. Final polishing was performed with a felt disc and 0.5-µm diamond paste for 10 minutes. The specimens were washed in running tap water, identified, and properly stored in recipients with moist gauze.

MICROHARDNESS ANALYSIS

Mean microhardness Knoop values were tested in a HMV Micro Hardness Tester (Shimadzu, scientific instruments, U.S.A) using a 10-g load for 10 seconds in 5 linear points starting 10 µm from the cavity floor toward the pulp chamber wall. A 25-µm distance was left between each point, resulting in 5 measurement points at 10, 35, 60, 85, and 110 µm from the cavity floor. For each depth, 3 measurements were made: 1 at the center or at the deepest part of the cavity and 100 µm to the left and to the right of the first point. The average of these 3 measurements was used to estimate microhardness (Figure 1). A calibrated and blinded examiner performed all measurements.

The remaining dentin (distance in µm between the cavity floor to the pulp chamber wall) was measured to estimate similarities in all groups. The measurements of indentations and microhardness values were performed with the Software Newage Testing Instruments C.A.M.S. Testing System, installed in a computer, connected to the microhardness tester through the optic system with digital image transference (Genwac High Resolution).

Based on an accepted difference of 20 Knopp Number Hardness (KNH) among the 3 groups, a 95% confidence level, and a power of 80%, it was estimated that 9 to 12 teeth were required for the study. Mineral content was compared among groups using the Mann-Whitney U test. The microhardness values of carious, sound, and treated dentin were compared among groups using a 2-way analysis of variance (alpha=0.05).

RESULTS

Of the 23 successful treated teeth (Franzon et al.[12]), 18 were collected after exfoliation. This sample is 43% of the initial sample (Pinto et al.) as shown in Figure 2.

After checking the normality of distribution using the Kolmogorov-Smirnov Z test, CH and GP were compared and the *t* test revealed no statistically significant difference among microhardness measurements (Table 1). Considering similar performance of the treatments between the 2 capping materials (CH and GP), the treated groups were combined into 1 for analysis in the present study.

The cavity depth measurements showed no difference between the carious, sound, and treated groups (means=852, 921, 833 µm; P=0.108).

Microhardness values of the sound, carious, and treated groups, respectively, at depths of 10 µm (54.8, 12.5, 38.3), 35 µm (62.0, 13.0, 38.0), 60 µm (56.8, 12.3, 39.1), 85 µm (58.9, 13.0, 40.3), and 110 µm (55.7, 14.2, 42.0) were significantly different statistically ($P \leq 0.01$) among the 3 groups (Figure 3).

DISCUSSION

The significantly harder dentin microhardness findings in the incomplete caries removal (ICR) -treated teeth) after IPT compared to teeth with ICR and no IPT (negative control) confirmed rehardening of dentin in the remaining carious dentin.

Rehardening of teeth submitted to IPT was observed in the present study through microhardness analysis comparing. The treated group showed higher microhardness values than the carious, but lower than the sound dentin group. These teeth remained for 37 to 71 months in the mouths of children, demonstrating clinical and radiographic success as characterized by the absence of pain, fistula, swelling of periodontal tissues, tooth mobility not associated with root resorption, and periapical or furcation radiolucency. Moreover, the teeth were obtained for the laboratory study at the moment of exfoliation, which coincided with the patients' chronological ages.

Analysis of the present study demonstrates that all cavities showed more than 3 mm clinical depth and no statistical difference was found among the 3 groups when remaining dentin (distance from the cavity floor to the pulp chamber wall) was measured (means of 921±418, 852±408, and 833±432 µm for the sound, carious, and treated dentin, respectively; P=0.108). Thus, remaining dentin of all teeth from the in vivo part of the study and from the teeth with deep cavities preparation or partial caries removal of the in vitro part can be considered comparable (cavity depth).

In this study, teeth treated with CH and GP were placed in the same group if there was a small number of teeth in each group and they presented similar microhardness values (Table 1). Microhardness values were

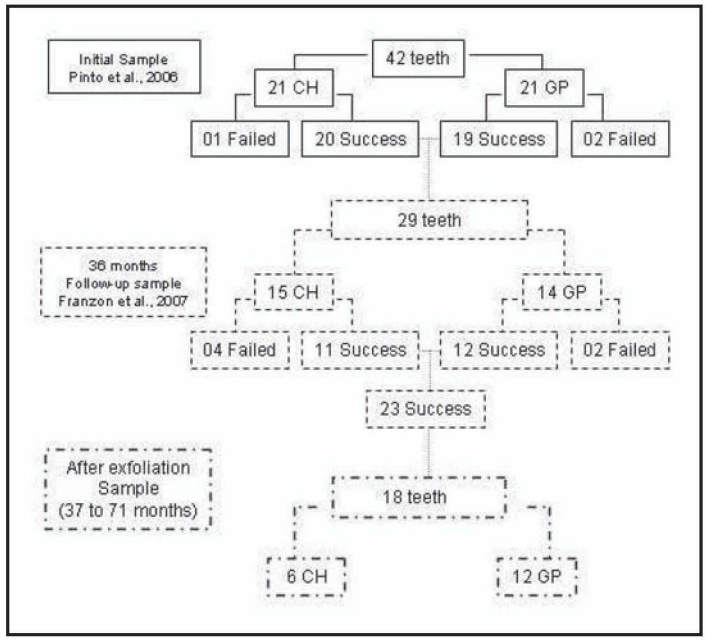

Figure 2. Histogram with sample composition.

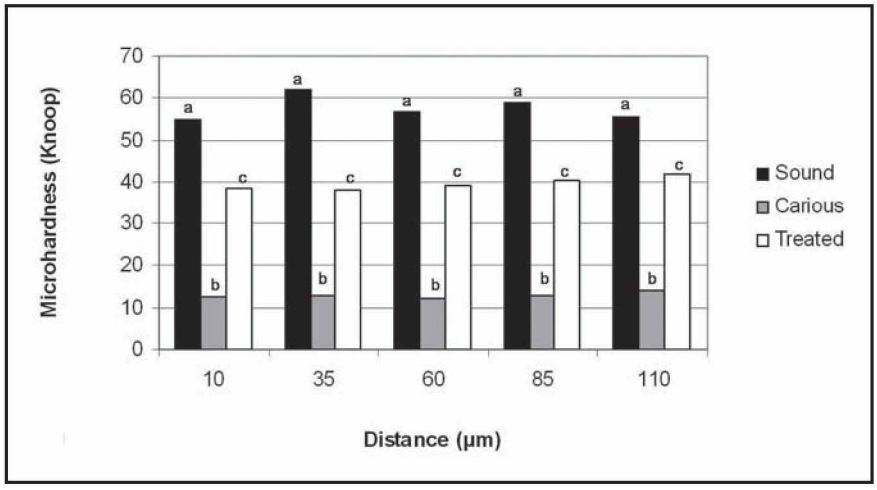

Figure 3. Microhardness values for all depths measured on sound, carious and treated dentin, showing that there was a statistically significant difference among the groups at all evaluated depths.

similar in all dentin depths from the surface to the cavity's deepest portion for the treated group (CH and GP). This finding suggests no influence of the material over the tissue. When different materials were used on demineralized dentin in vivo—including calcium hydroxide, zinc oxide/eugenol cement, glass ionomer cement, and adhesive systems—the tissue became harder and/or less infected. These alterations are due to treatment for the arrestment of the lesion in deciduous[9-13,21] and permanent teeth.[17,22-26]

In this study, the uniform hardness increase found in all dentin depths suggests that rehardening occurs independently of the use and type of the capping material. The effect is probably due to restorations of cavities with bonding materials (composite resin), which allows a satisfactory marginal sealing, making it difficult for the remaining micro-organisms to survive under substrate restriction and promoting dentin remineralization/sclerosis as a result of pulp reaction.

Similar to other data that compare CH with different materials such as glass ionomer[27] and adhesive systems,[10] this investigation does not demonstrate superior outcomes for CH when compared to GP. These data support the CH as a reliable alternative to IPT mainly because it is believed to be a remineralization inductive material.[9-11,17,24,25,27] This material also possesses bacteriostatic and bactericidal properties, although these characteristics have no influence on the present findings once it has been demonstrated that the physical presence of bacteria does not prevent repair or arrestment of cavitation.[9,13,24,25,28,29] In a study where total excavation was performed, complete elimination of bacteria involved in the caries process was not observed.[30]

The most common defense reaction of the dentin-pulp complex is dentin sclerosis. This consists of mineral deposition through the dentin tubules, resulting in gradual occlusion and promoting higher resistance to dentin.[31] In pathologic situations and restorative procedures, odontoblasts are stimulated to produce tertiary dentin through the expression of signaling molecules such as TGF-β.[32,33] Besides this, many investigations found that after partial removal of carious tissue and sealing of the cavity, there is a quantitative reduction of micro-organisms.[4,9,13,22,24,25,28]

The indication of IPT as a definitive technique in the deciduous dentition[4,9-12,15,16] can also be suggested by the clinical, radiographic, and laboratory findings from the present study. Reintervention or stepwise excavation of these teeth could have resulted in unnecessary pulp exposures. These originally acute caries patients should, however, be included in a periodic program of oral heath promotion and maintenance. They also should be monitored through clinical and radiographic follow-up and anamnesis data, avoiding reopening of the tooth until its exfoliation.

CONCLUSION

Based on this study's results, it can be concluded that rehardening of the carious dentin occurs when intentionally left under the restoration after indirect pulp treatment. This rehardening is probably a mineral gain resulting from the pulp's biological response and not exclusively from the capping material stimulation.

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