# Repairing Collagen in Dentin Carious Lesions. Influence of Sealing the Material: A Morphometric Study

Sérgio Luiz Pinheiro* / Paula Cristina Gallassi** / Talita Costa Saldanha***/
Iris Nogueira Bincelli**** / Pedro Paulo Barros***** / Gustavo Henrique Silva******

***Objective:*** *The aim of the present work was to morphometrically evaluate collagen in carious lesions sealed with calcium hydroxide, adhesive systems, glass ionomer cement, and an antibacterial cement.* ***Study design:*** *Samples of infected and affected dentin were stained with Sirius Red (SR). The areas intensively stained with SR were delimited, and the percentage of these areas was measured by blind calibrated examiners. The mean results were subjected to the Kruskal-Wallis test.* ***Results:*** *The affected dentin sealed with $Ca(OH)_2$ showed a better organization of the collagen in relation to the adhesive systems Prime & Bond ($p = .0159$) and Adhese ($p<0.0001$). The affected dentin sealed with Prime & Bond promoted better increase of organized collagen areas in relation to Adhese ($p = 0.0004$). The infected dentin sealed with glass ionomer cement ($p = 0.0018$) or antibacterial cement ($p = 0.0004$) brought a significant increase in the organized collagen areas.* ***Conclusions:*** *$Ca(OH)_2$ is indicated to seal affected dentin and glass ionomer cement and antibacterial cement may be used for treatment of infected dentin. The addition of antibiotics did not influence the restoration of the infected dentin.*

***Keywords:*** *dentin caries, minimal intervention, collagen, primary, deciduous teeth.*
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## INTRODUCTION

Currently pediatric dentistry supports the use of minimally invasive techniques for the treatment of carious dentin lesions. Among these treatment atraumatic restorative treatment (ART) and minimally invasive restorative dentistry are commonly used. In both techniques the removal of the infected dentin is done with sharp hand instruments, the affected dentin is maintained and the tooth is restored.[1]

The literature shows that hermetic sealing of carious dentin leads to a significant reduction in viable bacteria, paralyzes of carious lesions, and provides favorable conditions for dentin repair.[2] Therefore, several biomaterials with antibacterial and bacteriostatic properties are used.

Glass ionomer cements are used for ART because they adhere chemically to the dentinal structure. Several studies have shown microbial reduction and carious lesion cessation with the use of these materials.[3,4] The antibacterial properties of ionomer cements stimulate the reorganization of collagen cross-links present in the affected dentin. Some authors have defended the addition of antimicrobial substances to ionomer cements and the antibiotics have shown promising results.[4,5]

Calcium hydroxide $Ca[OH]_2$ has been extensively researched for indirect pulp capping because of its antimicrobial potential. Its alkaline pH as a result of a high concentration of hydroxyl ions leads to irreversible damage to the bacterial cell. This mechanism activates tissue enzymes promoting reorganization of collagen cross-links in the affected dentin.

The creation of resin tags, hybridization of carious lesions, and introduction of resin monomers with antibacterial properties in the adhesive systems promote favorable conditions for the reorganization of collagen cross-links in the affected dentin. The literature has evaluated the bond strength between sound and carious dentin, finding better

* Sérgio Luiz Pinheiro, PhD, Professor of Pediatric Dentistry, Pontifícia Universidade Católica de Campinas – PUC – Campinas
** Paula Cristina Gallassi, Undergraduate Student (FAPIC, REITORIA), Pontifícia Universidade Católica de Campinas – PUC – Campinas
*** Talita Costa Saldanha: Undergraduate Student (FAPIC, REITORIA), Pontifícia Universidade Católica de Campinas – PUC – Campinas
**** Iris Nogueira Bincelli: Undergraduate student, Pontifícia Universidade Católica de Campinas – PUC – Campinas
***** Pedro Paulo Barros: PhD and Professor of Physiology in Dentistry, Pontifícia Universidade Católica de Campinas – PUC – Campinas
****** Gustavo Henrique Silva: PhD and Professor of Histology in Dentistry, Pontifícia Universidade Católica de Campinas – PUC – Campinas

Send all correspondence to: Dr. Sérgio Luiz Pinheiro, Rua Raul Gasparini, 525, Bairro Panorama, Residencial Jardim das Palmeiras, Vinhedo – São Paulo – Brazil. CEP: 13280000.

Tel: 55 (19) 38864258
55 (11) 92450090

E-mail: psergio@usp.br or slpinho@puc-campinas.edu.br

bonding to sound dentin.[6] Higher bonding rates have been found in affected dentin compared with infected dentin.[7] Another line of research on the applicability of adhesive systems to carious dentin has focused on the role of metalloproteinase (MMP) in the durability of the hybrid layer. Some authors have affirmed that active MMPs in the carious substrate might degrade the hybridization of bonding agents.[8]

The aim of the present work was to morphometrically evaluate collagen in carious lesions sealed with calcium hydroxide $Ca[OH]_2$, adhesive systems, glass ionomer cement, and antibacterial cement.

## MATERIALS AND METHOD

This trial was approved by the Institutional Review Board of PUC-Campinas (Protocol 637/05). Fifteen samples of infected dentin and 12 of affected dentin were collected from deciduous molars at the Pediatric Clinic of PUC-Campinas. Differentiation between infected dentin and affected dentin was performed according to clinical criteria.[9] Infected dentin: wet, highly softened, with no resistance on removal; affected dentin: dried and resistant to mechanical removal, with the clinical appearance of "chips."

**Inclusion criteria:**

- Patients with carious dentin lesions without pulp involvement, characterized by the absence of radiolucent areas in the periapical and furcation area;
- Characteristic symptoms of reversible pulpal inflammation;
- Patients who agreed to participate in the trial and sign the informed consent.

**Exclusion criteria:**

- Carious lesions in dentin with pulpal involvement, characterized by periapical radiolucent areas;
- Teeth with characteristic symptoms of nonreversible pulpal inflammation;
- Teeth with clinical and radiographic signs of pulpal necrosis;
- Patients who did not agree to participate in the trial or did not sign the informed consent.

After medical history taking and clinical examination, X-rays were taken and hygiene and diet instructions were given. Then the patients with carious dentin lesions received local anesthesia with 2% lidocaine (Dentisply Probem, Catanduva, Brazil), prophylaxis with a Robinson brush (Microdont, São Paulo, Brazil) at slow speed, then a rubber dam (Madeitex, São Paulo, Brazil) was placed.

**Collection and sealing of the affected dentin samples:**

Twelve samples of affected dentin were collected from primary molars. The infected dentin (wet, highly softened, with no resistance to removal) was excavated from the teeth with the use of a curette; as soon as the carious dentin presented dry and resistant characteristics (appearance of chips) mechanical removal was interrupted. Approximately half the affected dentin was collected with a curette (Duflex) and the other half was sealed with different types of materials: $Ca(OH)_2$ (Dycal, Dentsply, São Paulo, Brazil), Prime & Bond adhesive system (Dentsply, São Paulo, Brazil), and Adhese self-etching adhesive (Ivoclar Vivadent, Liechtenstein, Germany).

**Collection and sealing of the infected dentin samples:**

Fifteen samples of infected dentin were collected from primary molars. Approximately half of the infected dentin (wet, highly softened, with no resistance to removal) was collected with a curette and the remaining half was sealed with Vidrion F glass ionomer cement (S.S. White, Rio de Janeiro, Brazil) or antibacterial cement (Fórmula & Ação, São Paulo, Brazil).

All materials were used according to the manufacturer's instructions (Table 1). All teeth, after carious lesions had been sealed, were restored with composite resin Z 100 (3M/ESPE, St Paul, Minn, USA).

After a 90-day period, the following procedures were conducted in all teeth: anesthesia with 2% lidocaine (Dentsply Probem, São Paulo, Brazil), prophylaxis with Robinson brush (Microdont) at slow speed, and the placement of rubber dam (Madeitex, São Paulo, Brazil). The restorations with composite resin and the sealing materials were removed, and excavation of the remaining dentin (infected and affected) was performed with curettes (Duflex-S.S. White, Rio de Janeiro, Brazil). Each tooth was restored with composite resin Z100 (3M/ESPE, St Paul, USA).

**Morphometric and statistical analysis**

A sound tooth having severe periodontitis was obtained, after written informed consent for the donation of human teeth. This sound tooth was used as a control (Figure 1). Samples of infected and affected dentin were stained with the SR and analyzed (×500) using the software, Tpsdig, version 1.38 (Rohlf FJ, Department of Ecology and Evolution, State University of New York, Stony Brook, NY). The vividly SR-stained areas (organized collagen) were delimited by blind calibrated examiners (Figure 2).[9] Results were

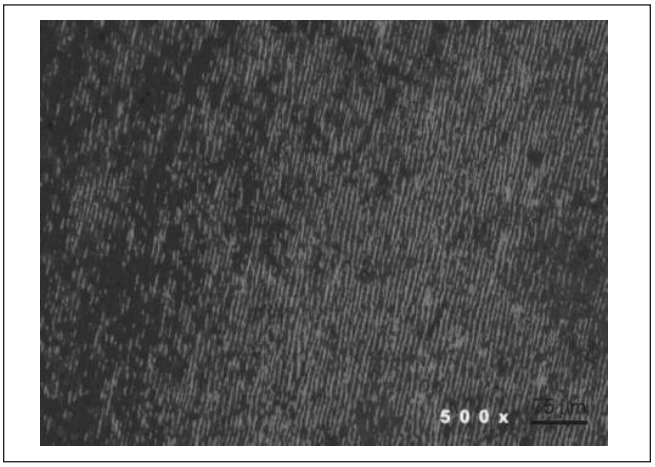


**Figure 1.** Sirius Red–stained sound dentin. Observe the absence of blackish areas that characterize the collagen organization.

**Table 1.** Sample groups

| Product | Manufacturer | Group | Classification | Lot | Composition | Substrate |
|---|---|---|---|---|---|---|
| **Dycal** | Dentsply São Paulo, Brazil | I | Calcium hydroxide | 93453 | Base paste: Disalicylate 1,3 – butylene glycol, zinc oxide, calcium phosphate, calcium tungstate, iron dioxide pigments, catalyzing paste: Calcium Hydroxide Sulphonamide N-ethil-o/p-toluene Zinc oxide, titanium dioxide, zinc stearate iron oxide pigments (only dentin color) | Affected dentin |
| **Prime & Bond** | Dentsply São Paulo, Brazil | II | One Bottle Adhesive System | 0235A | Di-trimethacrylate resins, amorphous sílica, penta, photoinitiators, stabilizers, hydrofluoride cetilamine and acetone. | Affected dentin |
| **Adhese** | Ivoclar Vivadent Liechenstein Germany | III | Self-etching Adhesive System | 60321 | AdheSE Primer, dimethacrylate, phosphonic acid acrylate, initiators and stabilizers in aquous solution. AdheSE Primer: HEMA, dimethacrylate, silicium dioxide, initiators and stabilizers. AdheSE DC Activator: initiators, solvents. | Affected dentin |
| **Vidrion F** | SSWhite, Rio de Janeiro, Brazil | IV | Glass Ionomer Cement | 00708 | Powder: Aluminum calcium sodium fluorosilicate, barium sulphate, polyacrylic acid, iron oxide pigment Liquid: tartaric acid, distilled water | Infected dentin |
| **Antibacterial Cement** | Fórmula & Ação, São Paulo, Brazil | V | Glass Ionomer Cement associated to Antibiotics | 07253 | Glass Ionomer Cement associated to 1% of metronidazol, 1% of ciprofloxacin, 1% of cefaclor | Infected dentin |

analyzed with the software Biostat 4 (Ayres M, Jr. MA, Ayres DL, Santos AAS, Pará University, Belém, Pará, Brazil). An intraclass correlation coefficient was used to evaluate the calibration among examiners, and the mean results were subjected to the Kruskal-Wallis test.

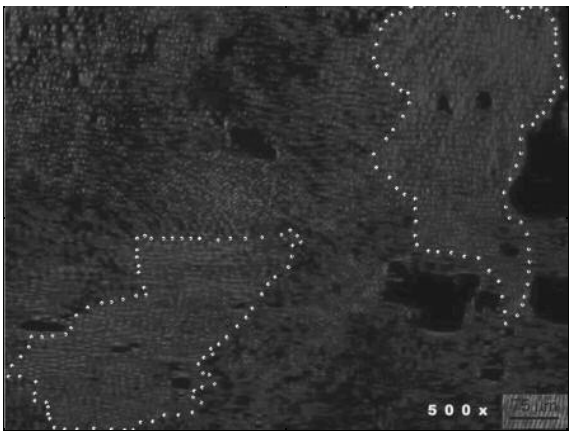


**Figure 2.** Measurement of Sirius Red–stained areas in the software Tpsdig. Delimited areas with dotted lines: regions with collagen similar to that of sound dentin (control) selected by blinded examiners.

## RESULTS

The examiners were calibrated (Table 2). Sealing of affected dentin with $Ca(OH)_2$ resulted in better collagen organization when compared with the adhesive systems Prime & Bond and Adhese, with significant statistical differences ($p = 0.0159$ and $p < 0.0001$, respectively). Among the adhesive systems, the sealing of affected dentin with Prime & Bond promoted a higher increase in organized collagen areas compared with Adhese, with statistically significant differences ($p = 0.0004$). Only Adhese failed to promote any increase in organized collagen areas after 90 days ($p = 0.6226$, Table 3).

Sealing of infected dentin with either glass ionomer cement ($p = 0.0018$) or antibacterial cement ($p = 0.0004$) for 90 days caused a significant increase in organized collagen

**Table 2.** Evaluation among examiners (organized collagen area)

| Variation among examiners | Experimental error | F | *P*-value | Intraclass correlation |
|---|---|---|---|---|
| .8260 | .0130 | 63.4109 | $P < .0001$ | .9689 |

**Table 3.** Arithmetic averages, standard deviations, and Kruskal-Wallis statistical analysis of organized collagen percentages after affected dentin sealing with calcium hydroxide, Prime & Bond, and Adhese

| Sample Groups ($n$ = 5) | Averages and standard deviations (pixels) | $P$-value | |
|---|---|---|---|
| Affected Dentin (DA)–1 | 32.37 (18.16)*,† | 1x2 ≤ .0001 | 2x3 = .0159 |
| DA + Calcium Hydroxide-2 | 77.34 (19.07)*,‡,≠ | | |
| DA + Prime & Bond-3 | 56.28 (28.14)†,‡, | 1x3 = .0118 | 2x4 ≤ .0001 |
| DA + Adhese-4 | 27.86 (13.22)≠, | | |
| | | 1x4= .6226 | 3x4= .0004 |

Groups that presented the same symbol (*,†,‡,≠, ): statistically significant different. Samples

areas. There was no statistically significant difference in the organized collagen of infected dentin after 90-day sealing with either glass ionomer cement or antibacterial cement ($p = 0.6381$, Table 4).

## DISCUSSION

**Table 4.** Arithmetic averages, standard deviations, and Kruskal-Wallis statistical analysis of organized collagen percentages after infected dentin sealing with glass ionomer cement and antibacterial cement

| Sample groups ($n$ = 5) | Averages and standard deviations (pixels) | $P$-value |
|---|---|---|
| Infected dentin (DI)–1 | 12.71 (11.00)*,† | 1x2 = .0018 |
| DI + Glass ionomer-2 | 37.28 (14.01)* | |
| DI + Antibacterial cement-3 | 45.72 (16.58)† | 1x3 = .0004 |
| | | 2x3= .6381 |

Groups that presented the same symbol (*,†): statistically significant different samples.

Advocates of minimally invasive restorative dentistry report that the maintainability of dentin affected by disease and methods of evaluating collagen after cavity restoration are very limited. Dental caries is a transmissible disease and has invasive and destructive features that lead to dental tissue loss.[4] The process of caries starts with the deposition of biofilm over the dental surfaces, especially on the occlusal ones.[10]

Carious lesions are characterized by demineralization of the inorganic portion and disorganization of the organic part, resulting in collagen denaturation. Some authors have reported the presence of two distinct layers in carious dentin. The most superficial region has a softened consistency and exhibits destruction of collagen-cross linking and demineralized intertubular dentin with irregularly scattered crystals. The deepest layer is hardened, with reversible collagen alterations and partially demineralized inter and peritubular dentin.[4-9,11-13] Banerjee *et al* (2000)[2] described six layers in carious dentin: (1) infected dentin, external layer, irreversibly demineralized; (2–4) affected dentin, reversibly demineralized, composed of external, transparent, and subtransparent layers; (5) sound dentin; and (6) a fine layer of dentin adjacent to the pulp tissue.

The efficient restoration of dental surfaces, physical removal of highly infected tissue, restriction of nutrients for bacteria, and use of bacteriostatic restorative materials are procedures that contribute to the interruption of caries progression. Nowadays dentistry offers treatment methods based on restorative materials with remineralization properties and antimicrobial action.[4]

Our results show that sealing of affected dentin with $Ca(OH)_2$ promoted higher organization of the collagen compared with the adhesive systems, Prime & Bond and Adhese, with significant differences. These findings agree with the studies[15,16] reporting $Ca(OH)_2$ as presenting antibacterial and bacteriostatic effects and the capability to induce dentin neoformation. This capability can be explained by the high concentration of hydroxyl ions that alter bacterial cell permeability, breaking down the organic compound structures and the supply of nutrients, causing toxic and harmful effects.[15,16] King, Crawford, and Lindahl[17] observed an 81.2% microbial reduction after carious lesions sealed with $Ca(OH)_2$ , Leung, Loesche, and Chabeneau[18] reported a 99.04% bacterial reduction and BjØrndal, Larsen, and Thylstrup[19] showed a 90% microbial reduction. They also reported increased hardening, darker color, and dry aspect of dentin after the experimental period. BjØrndal and Larsen[20] observed significant reduction in the number of anaerobic Gram-negative *Lactobacilli*, and a complete absence of *Prevotellae* and *Porphyromonas* after a carious lesion was sealed with $Ca(OH)_2$ from by 6 months. Maltz *et al* [21] reported 63.33% of zero anaerobic growth in carious lesions sealed with $Ca(OH)_2$. Some authors [19,21] observed that the lesions presented hardened consistency and dentin dryness after the experimental period. As for $Ca(OH)_2$ biocompatibility, Murray *et al* [22] reported a high stimulating capability to produce tertiary dentin and little reduction of the odontoblast layer.

In the present work, Prime & Bond promoted significant repair of affected dentin when comparing the areas before and after sealing. The organized collagen area in the affected dentin before sealing was 32.37%, which increased to 56.28% after a 90-day sealing period with Prime & Bond. This can be explained by the formation of an acid-resistant hybrid layer[23] and by the sealing of the residual microbiota in the affected dentin. Another factor that can favor the interruption of caries evolution after sealing with Prime & Bond is the microbial capability related to these agents' chemical composition. Several authors[24] have evaluated the antibacterial effect of the three adhesive systems (Single Bond, Prime & Bond NT, and Excite) on *Streptococcus mutans*, *Streptococcus intermedius, Lactobacillus acidophilus, Prevotela oris, Prevotela bivia, Prevotela denticola, Porphyromonas endodontalis, and Clostridium ramosum.* Prime & Bond NT effected antibacterial action against all bacteria. This adhesive system possesses a resin monomer that promotes adhesion and contributes to antibacterial action.

Researchers[24,25,26] observed that the one-bottle systems promote a significantly higher bond-resistance compared with the self-etching systems after 24 hours and 6 months.

This superiority reported in the literature justifies our results, which presented repair of the affected dentin collagen only when it was sealed with Prime & Bond.

Some authors[27] have observed that there is no difference in the adhesive resistance between affected dentin and sound dentin, which disagrees with other researchers[6] who evaluated Prime & Bond's NT and Excite's adhesive resistance and observed that adhesion in the sound dentin was higher than that of affected carious dentin.

Another substrate evaluated in this work was infected dentin. Ninety-day sealing with glass ionomer cement or antibacterial cement caused a significant increase of the organized collagen area. The fact that justifies these results is the antimicrobial capability of these two materials. Van Amerogen (1996)[28] reported that, among the dental materials used to seal carious lesions, glass ionomer cements are frequently mentioned in the literature, and their effects on the cariogenic microbiota are generally associated with release of the fluoride ion, aluminum, and the low pH. Additionally, he observed a higher inhibitor effect of the ionomer cements over *Streptococcus sanguis* in relation to *Streptococcus mutans*. Barkhordar *et al,*[29] and Van Amerogen[28] disagree with Loyola-Rodriguez, Garcia-Godoy, and Lindquist[30] that pH alterations of glass ionomer cements have antibacterial effects and credit the bacterial inhibition of this material to the liberation of fluoride ions. Herrera *et al* (1999)[31] and others[29,28] studied glass ionomer cement's bacterial activity and observed that cements inhibit bacterial growth. Mount[32] added that the antimicrobial capacity of ionomer cements may be associated with the adhesion of these materials to the cavity through the ionic change layer and the hermetic sealing o carious dentin lesions.

Massara, Alves, and Brandão (2002)[33] and Toi, Bönecker, and Cleaton-Jones (2003)[34] observed bacterial reduction after sealing of dentin affected by carious lesions with conventional glass ionomer cement in ART. The intertubular dentin was dense, with compact arrangement of collagen fibers and calcium concentration increasing from 30% to 48%. In our results, there was an increase from 12.71% to 37.28% in organized collagen areas, showing that significant microbial reduction after infected dentin sealing with ionomer cement promotes dentin repair due to reorganization of collagen fibers.

Infected dentin sealing with antibacterial cement brought about an increase from 12.71% to 45.72% in organized collagen areas in the infected dentin. Pinheiro *et al* [4] observed that the combination of antimicrobial agents such as metronidazole, ciprofloxacin, and ceflacor with glass ionomer cement significantly reduces the microbiota of infected dentin (average reduction, 98.65%). According to Pinheiro *et al*,[4] metronidazole is effective against cocci and anaerobic bacilli, ceflacor acts against Gram positive and Gram negative aerobic bacteria, and ciprofloxacin is efficient against Gram negative cocci and mycobacteria. Based on the fact that dentinal caries microbiota contain a predominance of facultative and obligate bacteria,[35] Gram negative bacteria,[36] and Gram positive cocci and rods,[37] the need to associate microbial agents that will cover the whole spectrum of dentinal caries microbiota remains.

Hoshino *et al* (1988)[38] and (1989)[39] reported that metronidazole reduces carious lesion bacteria in over 99% and that no contamination was found in dentin carious lesions that were covered for 1 day, 1 month, 1 year, or 2 years with -tricalcium phosphate cement combined with 8.5 mg of metronidazole. These researchers emphasized that metronidazole displayed an ample bacterial spectrum against anaerobic bacteria, agreeing with Imazato *et al*[40] Conversely, Sato *et al* [41] stated that metronidazole by itself, even in concentrations of 100 g/mL, is unable to eliminate all carious lesion bacteria, corroborating with Kudou *et al*,[42] who reported little metronidazole action against *Streptococcus sanguis*, *Streptococcus salivarius,* and *Actinomyces naeslundii*. Thus, Sato *et al*[41] proposed the combination of other antimicrobial agents with metronidazole to sterilize the lesions and observed that *in situ* sealing of the affected dentin with 1% metronidazole, 1% ciprofloxacin, and 1% ceflacor combined with -tricalcium cement for 24 hours exerted total antimicrobial action over carious lesion bacteria. An important point to be observed in the present work is that the addition of antibiotics to glass ionomer cements caused an increase of organized collagen areas, even though there were no significant differences concerning pure ionomer cement. Sealing with antibacterial cement resulted in areas containing 45.72% organized collagen and 37.28% with pure glass ionomer cement. This probably means that the greater microbial reduction effected by antibacterial cement does not influence the reparative process of carious tissue.

Based on the results, we have determined that $CA(OH)_2$, glass ionomer cement, and antibacterial cement are indicated for minimum intervention in pediatric dentistry. New studies must be conducted to evaluate the response to the dentin-pulp complex after sealing with different dental materials and observing the dentin repair process.

## CONCLUSIONS

1. $Ca(OH)_2$ and the Prime & Bond adhesive system are indicated for affected dentin sealing; glass ionomer cement and antibacterial cement can be used for treating infected dentin.
2. The Adhese self-etching adhesive system did not promote any dentin repair after sealing affected dentin for 90 days.
3. The addition of antibiotics did not influence infected dentin repair.

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