FINAL REPORT

TITLE:

Pulp response after application of Riva Light Cure and Riva Self Cure glass-ionomer cements in deep cavities prepared in human teeth.

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ABSTRACT

Objective: The aim of this *in vivo* study was to evaluate the dental pulp response after restoration of deep cavities with the resin-modified glass-ionomer cements (RMGIC) Riva Light Cure or Riva Self Cure (SDI, Bayswater, Victoria, 3153, Australia). **Methods:** Deep Class V cavities were prepared on the buccal surface of sound premolars scheduled to be extracted for orthodontic reasons. After cavity preparation, the cavities were restored with the following dental materials: Group 1 – Riva Light Cure; Group 2 – Riva Self Cure; and Group 3 (Control) - Dycal® (Dentsply Caulk, Milford, DE, US). Four additional teeth were used as an intact control group (Group 4). The dental materials used in this investigation were prepared according to manufacturer's instructions. After 7 or 30 days the teeth were extracted and processed through H&E, Masson´s trichrome and Brown & Brenn staining techniques. The stained sections were evaluated under light microscopy and the following histological events were scored according to the intensity of pulpal response: 1) Inflammatory cell response; 2) Tissue disorganization; 3) Bacteria presence; and 4) Tertiary dentin formation. The remaining dentin thickness (RDT) between the cavity floor and pulp tissue were measured for each tooth using a light microscope connected to a computer. **Results:** At 7-day period, it was observed that both RMGICs caused mild inflammatory pulp response associated with slight tissue disorganization. However, moderate inflammation was observed in one sample restored with Riva LC. Bacteria were evidenced in only one sample of Group 1 in which there was no significant pulp damage. Lack of tertiary dentin deposition occurred in all experimental and control groups at 7-day period. In Groups 1 and 2, the mean value of the RDT was 405.2µm and 447.8µm, respectively. At 30-day period, mild inflammatory pulpal response and tissue disorganization was observed in only two samples of Group 1. In this experimental group, deposition of tertiary dentin matrix was seen in two samples. Pulps with no inflammatory response were observed in Groups 2 (Riva Self Cure) and 3 (control – Dycal). In Groups 1 and 2, the mean value of the RDT was 392.6µm and 400.4µm, respectively. **Conclusion:** In general, Riva Light Cure caused more damage to the pulp tissue than Riva Self Cure. However, the discrete initial damage caused by both RMGICs decreased with time.

KEYWORDS: Resin-modified glass-ionomer cement, dentin, deep cavity, pulp, biocompatibilty.

CLINICAL RELEVANCE

Several dental materials have been recommended for indirect pulp capping, including conventional glass-ionomer cements (GICs) and resin-modified glass-ionomer cements (RMGICs) which were developed from the combination of conventional GIC formulation with polymerizable hydrophilic monomers of resin-based materials. It has been demonstrated that when used as cavity liners, the GICs ou even RMGICs may provide adequate protection to the dental pulp, preventing the occurrence of postoperative sensitivity. Particularly for the RMGICs, this characteristic is due in part to their self-adhesive nature and capacity for reduction of stress generated by composite resin polymerization shrinkage. Adhesion of RMGICs to dental substrates combined with their ability to release fluoride, which may act to prevent secondary caries, makes these materials an excellent option for use in different clinical procedures. In the last decades, some studies demonstrated the lower toxic effects of conventional self cure GICs when compared to the light cure RMGICs. Different chemical composition and techniques of polymerization of GICs as well as the variable pre-treatment of the cavity walls, such as application of acidic primers on dentin may increase the cytotoxic effects of this kind of dental material. The presence of organic monomers in the RMGICs may produce adverse biological reactions, such as local and systemic toxicity, pulpal reactions and allergic and estrogenic effects. Regarding pulpal reactions, it is known that due to its low molecular weight and high hydrophilicity, HEMA, which is present in many current RMGICs such as Riva Light Cure, may diffuse through dentin and reach the pulp tissue to cause local damage. Although some characteristics and properties of current GICs and RMGICs used in dentistry have been widely investigated, there were no research-based data on the pulpal effects of Riva Self Cure (GIC) and Riva Light Cure (RMGIC) when applied as liners in deep cavities prepared in human teeth. Then, the data obtained in this in vivo

study demonstrated that Riva Light Cure causes higher persistent damage to the pulp tissue than Riva Self Cure which, in turn, presents biocompatibility similar to calcium hydroxide cement - Dycal (gold standard dental material recommended by ISO, FDI and ANSI-ADA to be used for evaluating the biocompatibility of new liners). Therefore, in spite of both glass-ionomer cements exhibit low cytotoxic effects, Riva Self Cure should be considered the first choice material to line very deep cavities. This recommendation is based upon the biocompatibility results obtained in this investigation. However, further studies are needed to evaluate other properties of both glass-ionomer cements.

MATERIALS AND METHODS

Thirty caries-free human premolars in functional occlusion and scheduled to be extracted for orthodontic reasons were selected from young patients. The mean age of the patients was around 14 years. The parents/guardians as well as the volunteers, after reading and receiving all necessary explanations including the experimental rationale, the clinical procedures and possible risks, were asked to sign a consent form explaining the research protocol.

The radiographs taken for orthodontic treatment were also used to evaluate the possible presence of proximal caries or potential periapical pathology. As a common diagnostic procedure for tooth extraction, periapical radiographs were taken immediately before the extraction of each tooth.

Asepsis of the oral cavity was performed with a 0.12% chlorhexidine rinsing solution prior to administration of local anesthesia. After the tooth was cleaned with rubber cup and pumice slurry, buccal Class V cavities were prepared using a high-speed handpiece with copious water-cooling. In order to standardize the cavity to a preset depth, a slightly tapered diamond bur, with its cutting area previously limited to 2.5mm by means of a resin cap was used. The bur was replaced after every fourth cavity preparation to avoid excessive heating. The final dimensions of the buccal cavities was 3.0mm in length, 2.5mm deep, and 1.5mm wide with no undercuts (Figure 1).

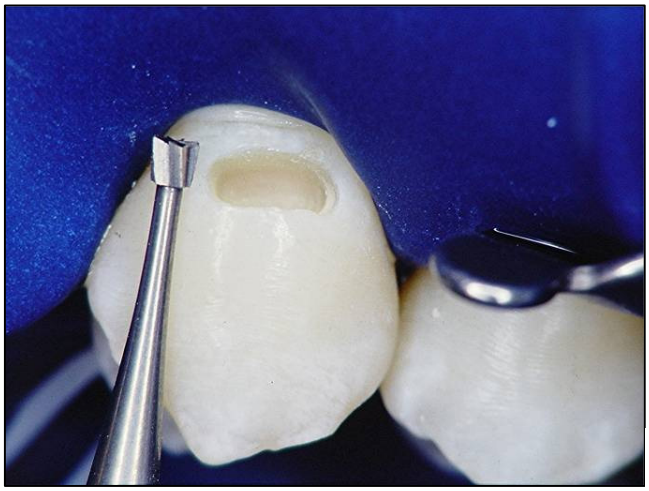

Figure1. Class V preparation on the buccal surface of an upper premolar

The teeth were divided into four Groups (Table 1) such that four sound teeth with no cavity preparation were included as an intact Control Group.

Table 1. Number of teeth according to groups and periods.

Groups	Periods		Total
	7 days	30 days	
Group 1 (Riva Light Cure)	5	5	10
Group 2 (Riva Self Cure)	5	5	10
Group 3 (control) (Dycal)	3	3	6
Group 4 (control) (intact)	2	2	4
Total	17	17	30

The manufacturers and chemical composition of the resin-modified glass-ionomer cements and the hard setting calcium hydroxide are described in Table 2.

In Groups 1 and 2, the cavities were restored with Riva Light Cure (SDI) or Riva Self Cure (SDI), respectively. For both of these groups, the cavity walls were etched with the conditioner provided by the manufacturers. Then, the cavities were rinsed thoroughly with water for 30 seconds in order to remove the conditioner agent. The cavity walls (dentin) were gently dried with sterilized absorbent paper. All cavities were restored to the enamel cavosurface margin with the Riva Light Cure or Riva Self Cure, which were

applied in increments of 1.5mm. Each increment of Riva Light Cure was light-cured for 20 seconds. A radiometer (Demetron/Kerr – Model 100P/N 10503, Danbury, CT, USA) was used to check the curing light intensity immediately prior to each clinical procedure. The light intensity was 470mW/cm^2. When necessary, any excess material at the cervical margin was mechanically removed using a fine grit diamond bur at high speed. After 24 hours, the restorations were finished and polished using fine diamond burs and mounted abrasive stones.

In Control Group 3, before restoration of the cavities with Riva Light Cure (SDI), the cavity floor was lined with the calcium hydroxide hard setting cement - Dycal. The following restorative procedures were carried out as previously described.

Table 2. Product details

Product	Manufacturer	Description	Composition
Riva Light Cure (Group 1)	SDI Bayswater, Victoria, 3153, Australia	Resin-modified glass-ionomer cement	Powder: Radiopaque fluoroaluminosilicate glass, polymerization initiator and pigments. Liquid Polycrilic acid, 2-hydroxyethyl methacrylate (HEMA), water, tartaric acid, stabilizers and polyemerization initiator.
Riva Self Cure (Group 2)	SDI Bayswater, Victoria, 3153, Austrália	Resin-modified glass-ionomer cement	Powder: Radiopaque fluroaluminosilicate glass, polyacrylic acid, pigments. Liquid: Polyacrylic acid, tartaric acid, and water.
Dycal (Group 3)	DENTSPLY Caulk Milford, DE, USA	Hard-setting calcium hydroxide liner	Base paste Calcium tungstate, zinc oxide, disalicylate ester of 1,3 butylene glycol. Catalyst paste Calcium hydroxide, zinc oxide, titanium dioxide.

At seven or 30 days after the clinical procedures a new radiograph was taken and the tooth was extracted under local or regional anesthesia. The roots were immediately sectioned midway between the CEJ and the root tip with a high-speed handpiece under water spray. The teeth were stored for 48 hours in formalin fixative solution at pH 7.2, decalcified in buffered Morse's solution, dehydrated, vacuum-infiltrated with wax paraffin and finally embedded in paraffin. Six-µm thick serial sections, mounted on glass slides, were stained with hematoxylin and eosin (H/E) and Masson´s trichrome. Bacteria were evidenced using the Brown & Brenn staining technique. As previously reported (Hebling et al., 1999, Costa et al., 2006, Costa et al., 2007) all sections were evaluated blind for five histological features according to defined criteria as given in Tables 3 through 6. The pulpal response was evaluated by light microscopy (Carl Zeiss 62774, Oberköchen, West Germany).

Table 3. Inflammatory cell response.

Score	Characterization
0	None or a few scattered inflammatory cells present in the pulp area corresponding to the axial wall, characteristic of normal tissue
1	Slight inflammatory cell infiltrate with polymorphonuclear (PMNs) or mononuclear leukocytes (MNLs)
2	Moderate inflammatory cell infiltrate involving the coronal pulp
3	Severe inflammatory cell infiltrate involving the coronal pulp or characterizing abscess

Table 4. Tissue disorganization

Score	Characterization
0	Normal tissue
1	Odontoblastic layer disorganized but central pulp normal
2	Total disorganization of the pulp tissue morphology
3	Pulp necrosis

Table 5. Stained bacteria

Score	Characterization
0	Absence
1	Presence of stained bacteria along the cavity lateral walls
2	Presence of stained bacteria along the cavity lateral walls and axial wall
3	Presence of stained bacteria along the cavity walls and within the cut dentin tubules

Table 6. Tertiary dentin formation

Score	Characterization
0	Absence
1	Modest hard tissue deposition beneath the axial wall
2	Moderate hard tissue deposition beneath the axial wall
3	Intense hard tissue deposition beneath the axial wall

The remaining dentin thickness (RDT) between the cavity floor and the pulp chamber (Figure 2) was measured for each tooth using a light microscope (Carl Zeiss) connected to a video-camera (Samsung Digital Camera – SSC/131, Samsung Electronics Co. Ltd., Korea).

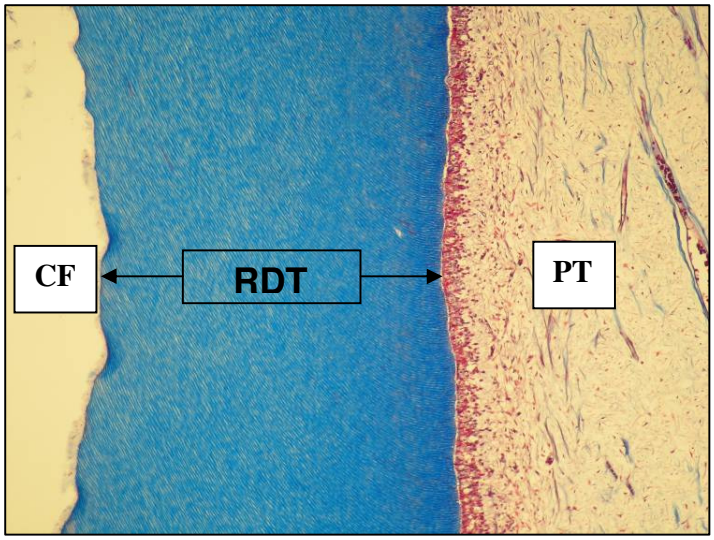

Figure 2. Histological section of a Class V preparation to evaluate the proximity to the pulp chamber (RDT = remaining dentin thickness; CF = cavity floor; and PT = pulp tissue)

The video images were loaded into a computer and processed using standard software (ImageLab, Softium Informática, São Paulo, Brazil).

RESULTS

During the experiment, the patients reported no particular symptoms or pain. The radiographic evaluation of all teeth used in the study demonstrated no periapical pathology before the clinical procedures. The radiographs taken immediately before extraction (7 or 30-day period) did not show any change in the periapical area of the restored teeth.

In two teeth, the pulp tissue was mechanically exposed during the cavity preparation. These teeth were excluded from the investigation in such way the two other premolars were used to replace them. The scores observed for every criterion according to groups and periods are shown in Table 7. The individual mean remaining dentin thickness (RDT) values as well as the period in which the teeth were subjected to the clinical procedures are presented in Table 8.

Table 7. Number of teeth for each score according groups and periods.

Histopatologic Event	Scores	Periods								Total
		7 days				30 days				
		0	1	2	3	0	1	2	3	
	Groups									
Inflammatory	1- Riva LC	1	3	1	0	3	2	0	0	10
cell response	2- Riva SC	2	3	0	0	5	0	0	0	10
	3- Ch	2	1	0	0	3	0	0	0	6
Tissue	1- Riva LC	1	4	0	0	3	2	0	0	10
Disorganization	2- Riva SC	2	3	0	0	4	1	0	0	10
	3- Ch	3	0	0	0	3	0	0	0	6
Bacteria	1- Riva LC	4	1	0	0	5	0	0	0	10
Presence	2- Riva SC	5	0	0	0	4	1	0	0	10
	3- Ch	3	0	0	0	2	1	0	0	6
Tertiary Dentin	1- Riva LC	5	0	0	0	3	2	0	0	10
Formation	2- Riva SC	5	0	0	0	4	1	0	0	10
	3- Ch	3	0	0	0	3	0	0	0	6

Table 8. Remaining dentin thickness (μm).

Group	Specimens	Periods	
		7 days	30 days
	1	372	602
	2	544	348
Riva	3	269	311
LC	4	461	407
	5	380	295
Mean		405.2	392.6
	1	621	418
	2	286	467
Riva	3	368	283
SC	4	452	505
	5	512	329
Mean		447.8	400.4
	1	508	377
CH	2	293	341
	3	348	489
Mean		383.0	402.3

Group 1 (Riva LC) / 7-day period

At 7 days, 3 samples exhibited discrete inflammatory response associated with slight tissue disorganization. In these samples the odontoblast layer related to the cavity floor was disrupted and a number of odontoblasts were displaced into the dentinal tubules (Figure 3A/B).

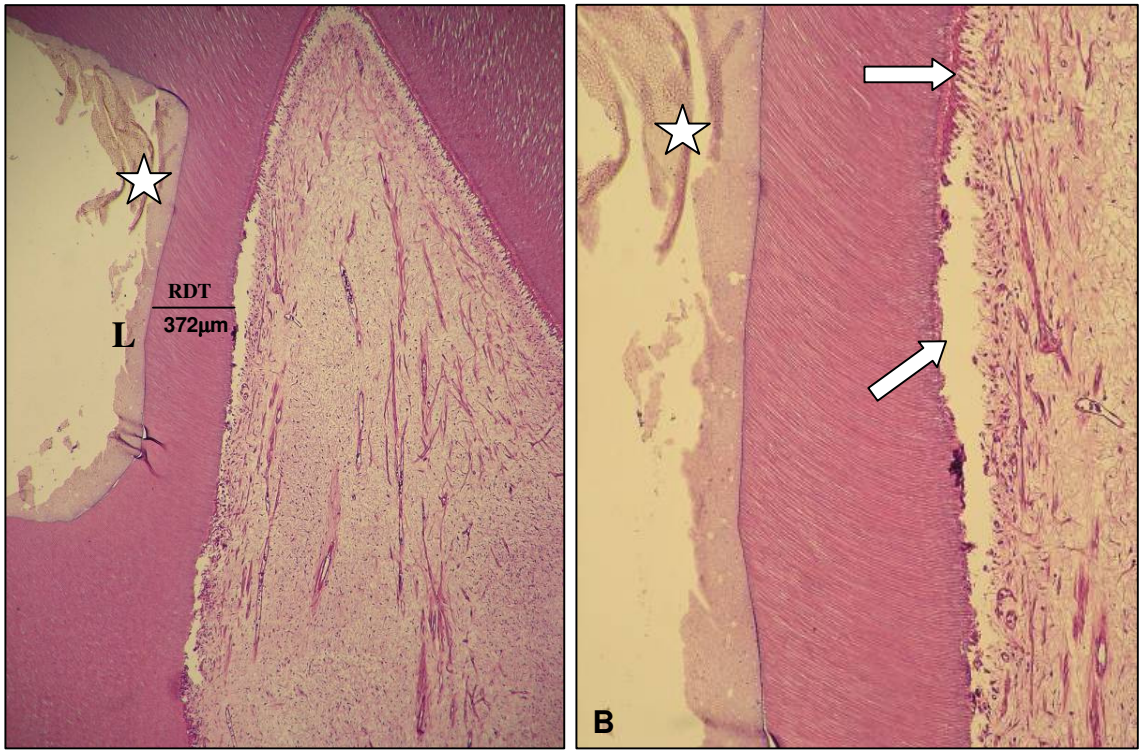

Figure 3. Group 1 (Riva LC, 7 days). A- Relationship between the Class V cavity, where the RMGIC (star) was applied (L) and the pulp (RDT = 372µm). H/E, 32x. Note the disruption of the odontoblast layer related to the cavity floor. B- Transition between the disrupted odontoblast layer (oblique arrow) related to the cavity floor and the intact subjacent odontoblast layer (horizontal arrow). H/E, 64x.

In one sample, deep tissue disorganization related to moderate inflammatory response mediated by mononuclear cells and presence of a number of small congested blood vessels was observed. Intense displacement of odontoblast cells into dentinal tubules was observed (Figure 4).

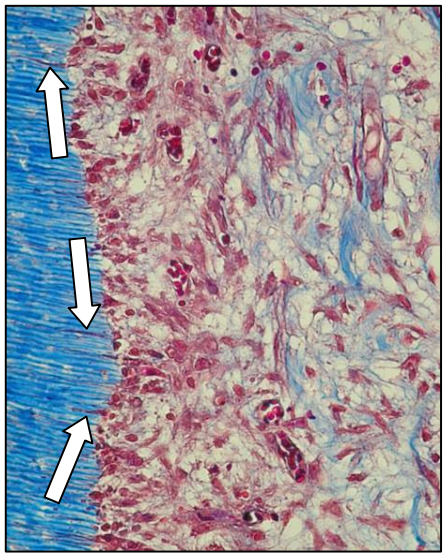

Figure 4. Group 1 (Riva LC, 7 days). The odontoblast layer is totally disrupted and several nuclei displaced into dentinal tubules are observed (arrows). Note the moderate inflammatory reaction mediated by mononuclear cells among several congested blood vessel adjacent to the dentin. Masson's Trichrome, 400x.

In 1 sample, in which the RDT was 544µm, no inflammatory response or tissue disorganization was observed (Figure 5A/B/C).

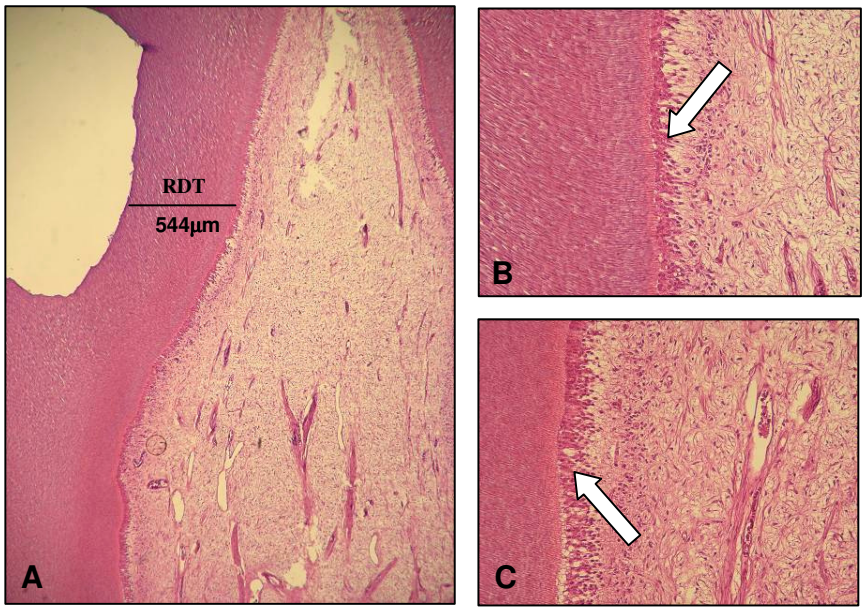

Figure 5. Group 1 (Riva LC, 7 days). A- The odontoblast layer related to the cavity floor is preserved (RDT = 544µm). H/E, 32x. B and C- Details of figure 5A. Note the continuous odontoblast layer (arrows). The subjacent tissue exhibits normal histological characteristics. H/E, 125x.

In this short period of evaluation (7 days), bacteria were evidenced in only one sample in which the RDT between the cavity floor and the pulp tissue was 380µm. However, such as occurred in other 2 samples free of microleakage, mild inflammatory pulp response and tissue disorganization was observed. Lack of tertiary dentin deposition was a common histological finding for all samples evaluated. The mean value of the RDT for this experimental group was 405.2µm.

Group 1 (Riva LC) / 30-day period

At 30 days, 3 samples exhibited no inflammatory pulp response. In these samples, a continuous odontoblast layer underlying the dentin tissue related to the cavity floor was observed. However, the number of odontoblasts was reduced when compared to the control groups (Figure 6A/B). Lack of tertiary dentin formation occurred in one of these samples in which no pulp tissue disorganization was seen (Figure 6A).

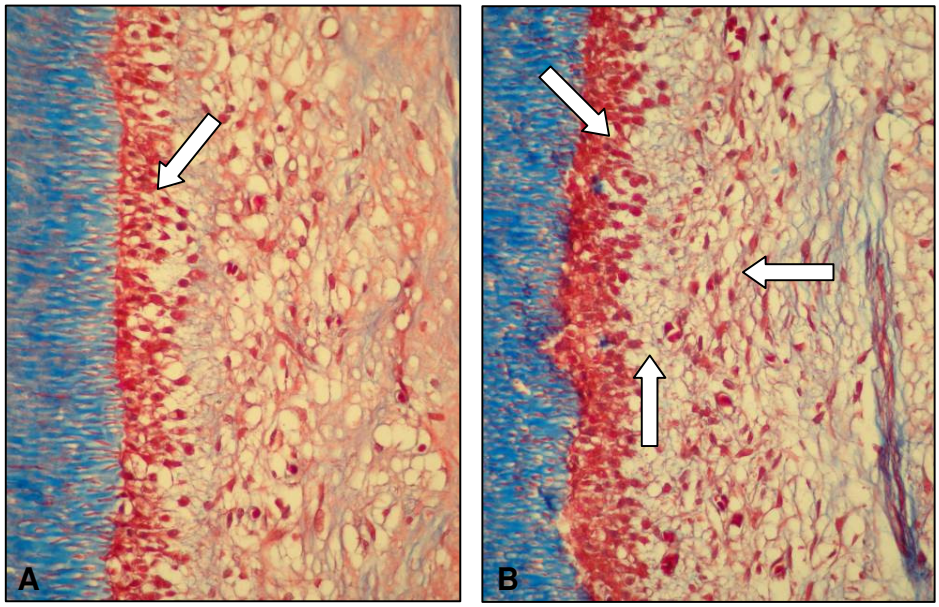

Figure 6. Group 1 (Riva LC, 30 days). A - No inflammatory response is observed. Note that the odontoblast layer is continuous, but the number of cells is reduced (arrow, RDT = 407µm). Masson's trichrome, 250x. B – In this intact control group (Group 4), the odontoblast layer (oblique arrow), cell-free zone (vertical arrow)and cell-rich zone (horizontal arrow) are preserved. Masson's trichrome, 250x.

Discrete inflammatory response and tissue disorganization characterized by lack of defined pulp tissue layers, such as cell-free and cell-rich zones occurred in two samples in which the RDT between the cavity floor and the pulp tissue was 295µm and 311µm (Figure 7).

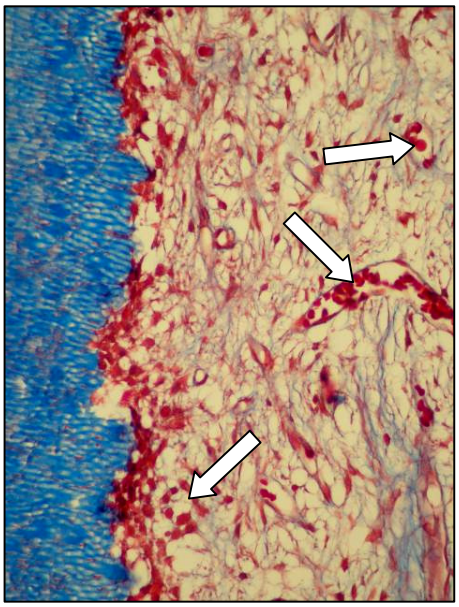

Figure 7. Group 1 (Riva LC, 30 days). A – Discrete persistent inflammatory response is observed in the pulp tissue related to the cavity floor. Note that the odontoblast layer, cell-free and cell-rich zones are not defined, characterizing the discrete local tissue disorganization in which a small number of mononuclear inflammatory cell as well as several congested blood vessels (arrows) are present (RDT = 295µm). Masson's trichrome, 250x.

In this 30-day period of evaluation, no microleakage was observed in all samples evaluated. Tertiary dentin deposition, characterized by discrete presence of tubular reactionary dentin matrix in the pulp area related to the cavity floor occurred in two samples (Figure 8). The mean value of the RDT for this experimental group was 392.6µm.

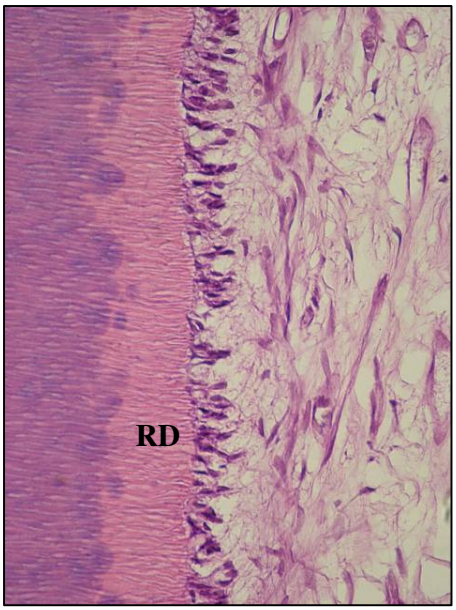

Figure 8. Group 1 (Riva LC, 30 days). No inflammatory response is observed. Note the deposition of a thin layer of reactionary dentin (RD) matrix in the pulp zone related to the cavity floor (RDT = 348µm). H/E, 250x.

Group 2 (Riva SC) / 7-day period

At 7 day-period, 3 samples presented mild inflammatory reaction mediated by mononuclear cells and a number of small blood vessels adjacent to the odontoblasts (Figure 9A/B/C). All these three samples exhibited slight disruption of the odontoblast layer related to the cavity floor as well as discrete pulp tissue disorganization.

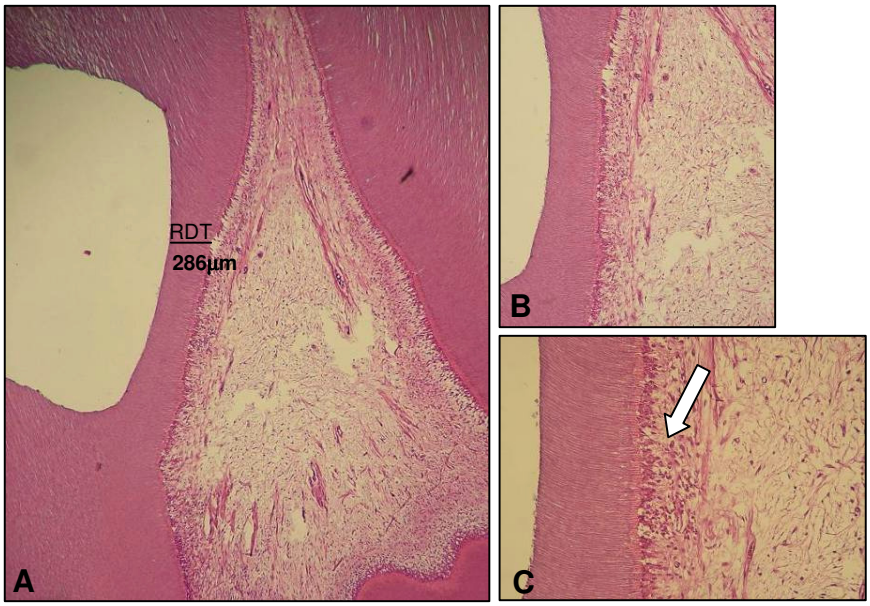

Figure 9. Group 2 (Riva SC, 7 days). A- The odontoblast layer related to the cavity floor is slightly disrupted, characterizing the discrete tissue disorganization (RDT = 286µm). H/E, 32x. B- Detail of Figure 6A. H/E, 64x. C- High magnification of Figure 6B. Note that the cell-free and cell-rich zones are disorganized (arrow). Mild inflammatory pulpal response mediated by mononuclear cells is observed. H/E, 250x.

Two samples, in which the RDT was 621µm and 512µm, exhibited no inflammatory response or tissue disorganization, characterizing the normal pulp tissue (Figure 10).

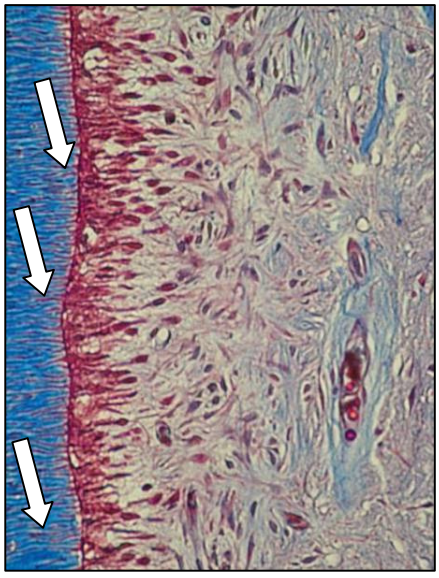

Figure 10. Group 2 (Riva SC, 7 days). A- The pulp tissue subjacent to the cavity floor presents normal histological characteristics (RDT = 512µm). Note that the cytoplasm processes from the odontoblast cells are intact within the dentinal tubules (arrows). Masson's trichrome, x 400x.

In all those histological sections stained with the Brown & Brenn technique, bacteria were not evidenced in the cavity walls. In addition, no tertiary dentin deposition occurred. The mean value of the RDT for this experimental group was 447.8µm.

Group 2 (Riva SC) / 30-day period

No inflammatory pulp response was observed in all samples evaluated. However, in two samples the odontoblast layer related to the cavity floor presented a reduced number of cells, especially when compared to the control group. In one of these two samples (RDT = 283µm), discrete tissue disorganization was observed (Figure 11).

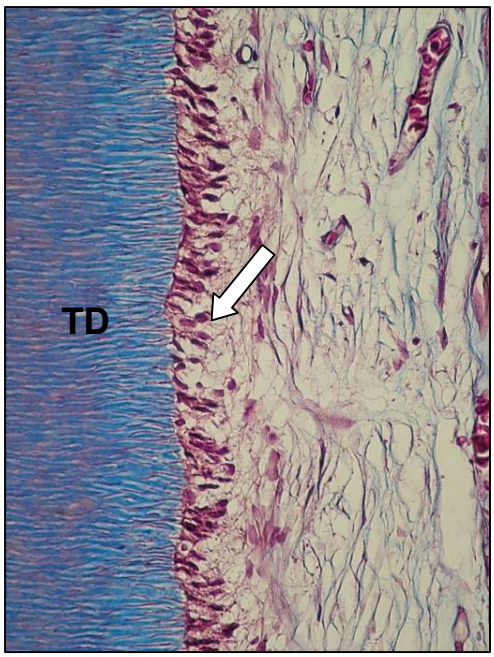

Figure 11. Group 2 (Riva SC, 30 days). The pulp tissue subjacent to the cavity floor presents discrete tissue disorganization, characterized by lack of defined cell-free and cell-rich zones. No inflammatory response is observed. Note the reduced number of odontoblasts (arrow) adjacent to the tubular dentin (TD) (RDT = 283µm). Masson's trichrome, x 400x.

In this experimental group, no microleakage occurred in four samples. However, histological sections obtained from only one sample exhibited bacteria at the lateral cavity walls but not inside the dentinal tubules. In this particular sample (RDT = 467µm) no

inflammatory pulpal response or significant tissue disorganization was observed (Figure 12). The mean value of the RDT for this experimental group was 400.4µm.

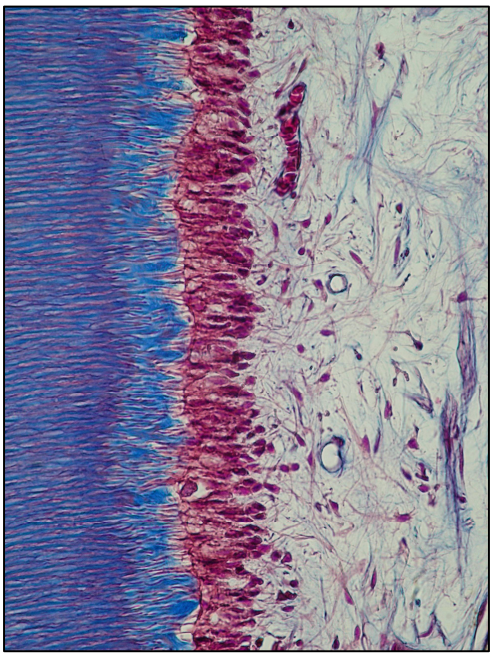

Figure 12. Group 2 (Riva SC, 30 days). The pulp tissue subjacent to the cavity floor presents continuous odontoblast layer (RDT = 467µm). Masson's trichrome, x 400x.

Group 3 (Calcium Hydroxide - Dycal) 7 and 30-day periods

At 7-day period, one sample in which the RDT was 293µm, exhibited slight disruption of the odontoblast layer related with the cavity floor as well as a number of mononuclear inflammatory cells and small blood vessels. However, the remaining pulp tissue exhibited normal histological characteristics (Figure 13).

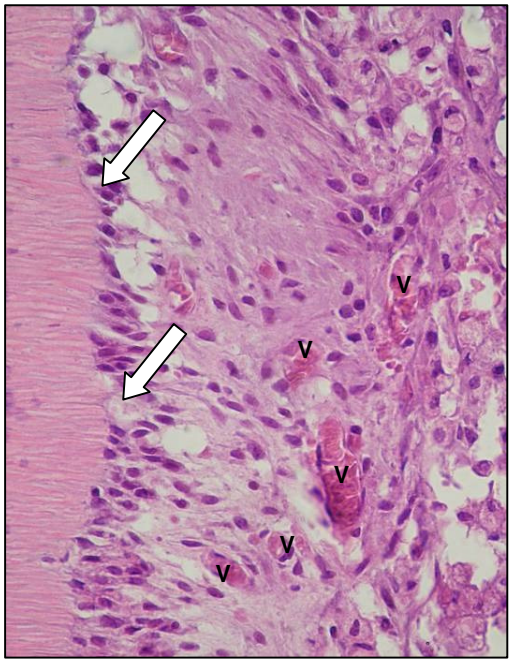

Figure 13. Group 3 (Dycal, 7 days). The pulp tissue subjacent to the cavity floor exhibits slight disruption of the odontoblast layer (arrows) (RDT = 293µm). Note the discrete disorganization of the adjacent pulp tissue in which mononuclear inflammatory cells among dilated and congested blood vessels (V) can be seen. H/E, 400x.

Another 2 samples of this experimental group as well as all samples evaluated at 30 days presented no inflammation or tissue disorganization. In only one sample of the 30-day period, bacteria were evidenced at the lateral walls of the cavity. However, in this sample as well as in all other samples of this group, the odontoblast layer related to the cavity floor was continuous with that one surrounding the coronary pulpal space and the cell-free and the cell-rich zones were preserved. The mean value of the RDT for the samples evaluated at 7 and 30 days were 383.0µm and 402.3µm, respectively. The normal histological findings were also observed in the intact control group which was used in the present investigation to assess the quality of the laboratory histological processing of the samples (Figures 6B and 14A/B).

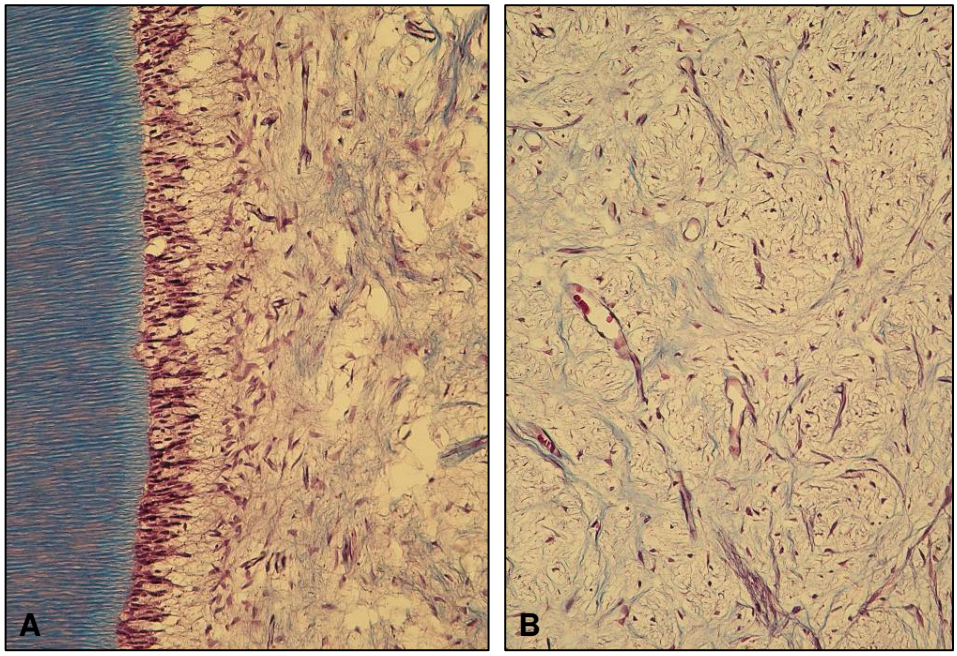

Figure 14. Intact Control Group. A- The pulp tissue of the sound premolar exhibits normal histological characteristics. Note the continuous and homogeneous odontoblast layer as well as the defined cell-free and cell-rich zones. Masson's trichrome, x 250. B- The central part of the pulp tissue also presents normal histological characteristics. Masson's trichrome, x 250.

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