Biocompatibility of a restorative resin-modified glass ionomer cement applied in very deep cavities prepared in human teeth

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This study evaluated whether a restorative resin-modified glass ionomer cement, Vitremer (VM), would be biocompatible with pulp tissue when used as a liner in very deep cavities prepared in young human permanent teeth. Two dental cements in current use as liner materials, Vitrebond (VB) and Dycal (DY), were compared to VM. Class V cavities were prepared in 36 sound premolars that were scheduled for extraction, and the cavity floor was lined with the restorative cement (VM) or a liner/base control cement (VB or DY). For VM specimens, the cavity floor was pretreated with a primer (polyacrylic acid plus 2-hydroxyethyl methacrylate). Teeth were extracted after 7 or 30 days and processed for microscopic evaluation. In the VM group, inward diffusion of dental material components through dentinal tubules, associated with disruption of the odontoblastic layer, moderate to intense inflammatory response, and resorption of inner dentin, was observed in 2 teeth at 7 days. These histologic features were observed in 1 tooth at 30 days. In the VB group, mild inflammatory reactions and tissue disorganization observed at 7 days were resolved at 30 days. No pulpal damage occurred in the DY specimens. Of the materials tested, only Vitremer was not considered biocompatible, because it caused persistent pulpal damage when applied in very deep cavities (remaining dentin thickness less than 0.3 mm).

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The high density of large dentinal tubules in very deep dentin characterizes the significant permeability of this dental substrate. This wet environment interferes with the degree of polymerization of resin-based dental materials, which may facilitate the inward diffusion of free unreacted toxic components across dentin to reach the pulp chamber.[1-3] Therefore, for pulpal damage to be prevented, the use of biocompatible liners has been recommended before the adhesive restoration of very deep cavities.[3]

Conventional glass ionomer cements (GICs) have been widely used as liners because of their acceptable mechanical and physical properties, long-term antibacterial activity, chemical adhesion to tooth structures, and biocompatibility.[4,5] The incorporation of resin monomers in GIC compositions, giving rise to resin-modified glass ionomer cements (RMGICs), improved the mechanical properties of this type of hard-setting material. RMGICs provide a new range of options for clinical applications, including full restorations in pediatric dentistry. The presence of a resin matrix in the RMGICs also allows the adhesion of these materials to composite resins, making these resin-based cements adequate for use as a liner or a base for the adhesive restoration of cavities prepared in permanent teeth.[6]

In a 2011 study, Costa et al evaluated the biocompatibility of a specific RMGIC, Vitrebond (3M ESPE).[7] The authors demonstrated that this resin-based, light-cured cement caused no significant alterations in pulp tissue of human premolars when applied as a liner in very deep cavities with a remaining dentin thickness (RDT) of less than 0.3 mm. While there was a slight inflammatory reaction and disorganization of the odontoblastic layer 7 days after the clinical procedures, it had reversed 30 days later.[7] However, as a dental material, Vitrebond is only recommended to be applied as a liner or base product.

Another RMGIC, Vitremer (3M ESPE), which is indicated for direct restoration and core build-up, has been widely used in pediatric dentistry, especially for children who need special care.[8,9] This use of an RMGIC requires previous application of a light-cured acidic primer containing 2-hydroxyethyl methacrylate (HEMA) to the dentin substrate. It is known that the treatment of deep dentin with acidic agents prior to the application of resin-based materials may damage pulp tissue.[10] Therefore, the aim of the present study was to evaluate the biocompatibility of Vitremer applied in very deep cavities prepared in human teeth. The null hypothesis was that there would be no difference concerning the biocompatibility of

Vitremer, Vitrebond, and Dycal (Dentsply Sirona), which is a hard-setting calcium hydroxide cement (CHC), when these dental materials were applied to the floor of very deep cavities.

Materials and methods

Thirty-six caries-free human premolars in functional occlusion (scheduled to be extracted for orthodontic reasons) were selected from young patients. The mean age of the patients was approximately 16 years. After receiving all necessary explanations about the research protocol, experimental rationale, clinical procedures, and possible risks, the parents or guardians and volunteers read and signed an informed consent form. This study was carried out in accordance with the Code of Ethics of the World Medical Association's Declaration of Helsinki.[11]

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

The 36 teeth were divided into 3 experimental groups of 12 each: Vitremer (VM), Vitrebond (VB), and Dycal (DY). The cavity floor of each tooth was lined with one of the materials. Both VB and DY were included as control materials, since previous studies demonstrated that both cements are biocompatible when used to line very deep cavities prepared in human teeth.[7,10] Two additional sound teeth were included as intact controls to assess the quality of the laboratory processing of teeth that received cavity restoration. The chemical compositions of the products used in this study are shown in Table 1.

Table 1. Dental materials used in the study.

Dental material	Description	Main components
VM	Light-cured RMGIC	**Primer:** HEMA, ethyl ethanol, polycarboxylic acid, initiators **Liquid:** Resin-modified polyalkenoic acid, HEMA, initiators (including camphorquinone) **Powder:** HEMA, Bis-GMA, water, initiators, and a radiopaque FAS glass
VB	Light-cured RMGIC liner/ base	**Powder:** Zinc radiopaque FAS glass **Liquid:** Modified polyacrylic acid with pendant methacrylate groups, HEMA, photoactivator, water
DY	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

Abbreviations: Bis-GMA, bisphenol A glycidyl methacrylate; DY, Dycal; FAS, fluoroaluminosilicate; HEMA, 2-hydroxyethyl methacrylate; RMGIC, resin-modified glass ionomer cement; VB, Vitrebond; VM, Vitremer.

Asepsis of the oral cavity was performed with a 0.12% chlorhexidine rinse prior to administration of local anesthesia. After the teeth were cleaned with rubber cups and pumice slurry, buccal Class V cavities were prepared by means of a high-speed handpiece cooled with copious water spray. For standardization of the cavity to a preset depth, a slightly tapered diamond bur, with its cutting area previously limited to 2.5 mm by means of a resin cap, was used.[7,10] To avoid overheating, the bur was replaced after every fourth cavity preparation. The final dimensions of the buccal cavities were 3.0 mm long, 2.5 mm deep, and 1.5 mm wide, with no undercuts.

All dental materials were prepared in accordance with their manufacturer's instructions and applied strictly to the cavity floor; then, the cavity was restored with composite resin.[12-14] In the VM group, a brush was used to apply primer to dentin for 30 seconds. This was followed by gentle air drying for 15 seconds. The process was repeated once, and the primer was light cured for 20 seconds. (The light intensity was standardized at 420 mW/cm^2 during the experiment.)

The VM powder was fluffed in the jar by shaking. One level scoop of powder and one drop of liquid were dispensed onto the mixing pad. A cement spatula was used to mix the powder and liquid within 45 seconds. A thin layer of the mixed liner was applied to the cavity floor using a ball applicator and light cured for 40 seconds.

The 35% phosphoric acid etchant was applied to enamel for 30 seconds and to dentin for 15 seconds; the surfaces were then rinsed for 30 seconds. Excess water was blotted, leaving the tooth moist. Two consecutive coats of Adper Single Bond 2 (3M ESPE) adhesive were applied to etched enamel and dentin and agitated for 15 seconds, using a fully saturated brush tip for each coat. The adhesive was dried gently for 2 to 5 seconds and light cured for 20 seconds.

The cavity was restored with Filtek Z350 composite resin (3M ESPE) placed in increments of 2 mm. Each increment was light cured for 40 seconds. The restoration was finished and polished.

In the VB group, VB powder was fluffed in the jar by shaking. One level scoop of powder and one drop of liquid were dispensed onto the mixing pad. A small spatula was used to mix the powder and liquid together for 10 seconds. A thin layer of the mixed liner was applied to the cavity floor with a ball applicator and light cured for 30 seconds. The bonding and restorative procedures were the same as those described for the VM group.

In the DY group, equal amounts of the catalyst and base pastes were dispensed onto the mixing pad. A small spatula was used to mix the pastes together for 10 seconds. A thin layer of the mixed liner was applied to the cavity floor with a ball applicator. The bonding and restorative procedures were the same as those described for the VM group.

At 7 or 30 days after the clinical procedures, a new radiograph was taken, and the study teeth were extracted under local or regional anesthesia. Two patients in the DY group discontinued participation in the study before the 30-day evaluation. The roots were immediately sectioned midway between the cementoenamel junction and the root tip with a high-speed handpiece used under water spray. The teeth were stored for 48 hours in a formalin fixative solution at pH 7.2, decalcified in buffered Morse solution, dehydrated, vacuum infiltrated

Table 2. Histopathologic events and scores.[7,10]

Histopathologic event	Score			
	0	1	2	3
Inflammatory cell reaction	Normal tissue	Slight inflammatory reaction beneath the axial wall	Moderate inflammatory reaction involving the coronal pulp	Severe inflammatory reaction involving the coronal and radicular pulp, which may or may not be associated with abscess
Tissue disorganization	Normal tissue	Disorganization limited to odontoblastic layer	Disorganization involving the coronal pulp	Disorganization involving the coronal and radicular pulp, associated with necrotic areas
Reactionary dentin formation	Absent	Slight tertiary dentin deposition beneath the axial wall	Moderate tertiary dentin deposition beneath the axial wall	Intense tertiary dentin deposition beneath the axial wall
Stained bacteria	Absent	Stained bacteria at lateral walls	Stained bacteria at lateral and axial walls	Stained bacteria at cavity walls and within dentinal tubules

Table 3. Remaining dentin thickness (mm), by liner material and evaluation period.

Specimen	Material		
	VM	VB	DY
At 7 d			
1	0.347	0.512	0.563
2	0.291	0.351	0.228
3	0.399	0.333	0.290
4	0.524	0.249	0.392
5	0.506	0.296	0.434
6	0.242	0.471	0.495
Mean (SD)	0.385 (0.104)[a]	0.369 (0.093)[a]	0.400 (0.114)[a]
At 30 d			
1	0.617	0.199	0.344
2	0.348	0.451	0.495
3	0.377	0.457	0.212
4	0.417	0.409	0.291
5	0.221	0.311	*
6	0.306	0.413	*
Mean (SD)	0.381 (0.66)[A]	0.373 (0.119)[A]	0.335 (0.93)[A]

Abbreviations: DY, Dycal; VB, Vitrebond; VM, Vitremer.

*Two patients in the DY group discontinued participation in the study before the 30-day evaluation.

Means with the same uppercase or lowercase letter are not significantly different ($P > 0.05$; 2-way analysis of variance).

Table 4. Number of teeth assigned each event score, by liner material and evaluation period.

Histopathologic event	Material	Score at 7 d				Score at 30 d				Total
		0	1	2	3	0	1	2	3	
Inflammatory cell reaction	VM	1	3	1	1	3	2	1	0	12
	VB	2	4	0	0	5	1	0	0	12
	DY	4	2	0	0	4	0	0	0	10*
Tissue disorganization	VM	2	3	1	0	2	4	0	0	12
	VB	3	3	0	0	5	1	0	0	12
	DY	4	2	0	0	4	0	0	0	10*
Stained bacteria	VM	6	0	0	0	5	1	0	0	12
	VB	6	0	0	0	6	0	0	0	12
	DY	5	1	0	0	4	0	0	0	10*
Reactionary dentin formation	VM	6	0	0	0	5	1	0	0	12
	VB	6	0	0	0	5	1	0	0	12
	DY	6	0	0	0	4	0	0	0	10*

Abbreviations: DY, Dycal; VB, Vitrebond; VM, Vitremer.

*Two patients in the DY group discontinued participation in the study before the 30-day evaluation.

with wax paraffin, and embedded in paraffin. Subsequently, 6-µm-thick serial sections (40 per tooth) were obtained, mounted on glass slides, and stained with hematoxylin and eosin or Masson trichrome stain. The presence of bacteria was checked via the Brown & Brenn staining technique. Selected sections underwent a blind evaluation for 4 histologic features (Table 2).[7,10]

Ten sections of each tooth in each experimental and intact control group were evaluated. The pulpal response was evaluated by light microscopy (model 62774, Carl Zeiss Microscopy).

The same method performed in earlier studies was used to measure the RDT between the cavity floor and the pulp chamber for each tooth by means of a light microscope connected to a video camera (Samsung Digital Camera SSC/131, Samsung Electronics Co, Ltd).[7,10] The images were loaded into a computer and processed with standard software (ImageLab, Softium Informática). Data were analyzed statistically by 2-way analysis of variance (ANOVA) for the variables *material* and *period* at the preset significance level of 5% ($\alpha = 0.05$).

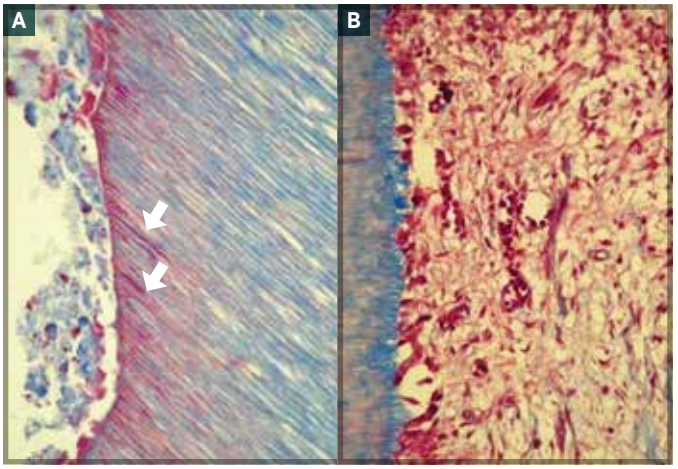

Fig 1. Tooth specimens from the Vitremer (VM) group at 7 days. A. Cavity floor on which VM was applied as liner. Note the inward diffusion of dental material through dentinal tubules (arrows). Remaining dentin thickness (RDT), 0.399 mm (Masson trichrome; original magnification 250×). B. The odontoblastic layer subjacent to the cavity floor is disrupted. Note the mild inflammatory reaction mediated by mononuclear cells associated with small, congested blood vessels (Masson trichrome; original magnification 250×).

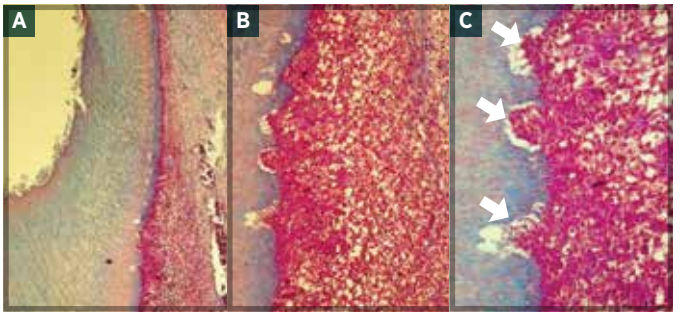

Fig 2. Tooth specimens from the VM group at 7 days. A. Relationship between Class V cavity and the pulp. RDT, 0.242 mm (Masson trichrome; original magnification 32×). B. Detail of Fig 2A showing the pulp area at the cavity floor. Note the complete disruption of the odontoblastic layer (Masson trichrome; original magnification 125×). C. High magnification of Fig 2B. Observe the intense local inflammatory cell infiltrate and sites of inner dentin resorption (arrows) (Masson trichrome; original magnification 250×).

Results

No pain or particular symptoms were reported by the patients during the study. Radiographic evaluation of the teeth demonstrated no periapical pathosis prior to the clinical procedures or extractions.

The mean RDT values associated with each dental material and evaluation period are shown in Table 3. Neither the individual factors (material and period) nor the interaction between them was statistically significantly different ($P > 0.05$; 2-way ANOVA). Consequently, there was no difference among the RDT values when materials and periods were compared. The scores obtained for each criterion according to the groups and evaluation periods are shown in Table 4.

VM group

At 7 days, 3 specimens (RDT, 0.347-0.506 mm) exhibited disruption of the odontoblastic layer associated with a mild inflammatory response in the pulp zone related to the cavity floor. In these specimens, the inflammatory reaction was mediated by mononuclear cells, and numerous small blood vessels were observed adjacent to the disrupted odontoblastic layer (Fig 1). A moderate inflammatory reaction was observed in 1 specimen (RDT, 0.291 mm). An intense inflammatory response associated with resorption of inner dentin occurred in another specimen, in which the RDT between the cavity floor and the subjacent pulp tissue was 0.242 mm (Fig 2). One specimen with an RDT of 0.524 mm exhibited no inflammatory response or tissue disorganization (Fig 3). In most of the specimens evaluated, either no or only discrete tissue disorganization was observed. Bacteria were not found in any of the histologic sections stained with the Brown & Brenn technique. No deposition of dentin matrix was observed in any of the specimens. The mean RDT for this experimental group was 0.385 mm.

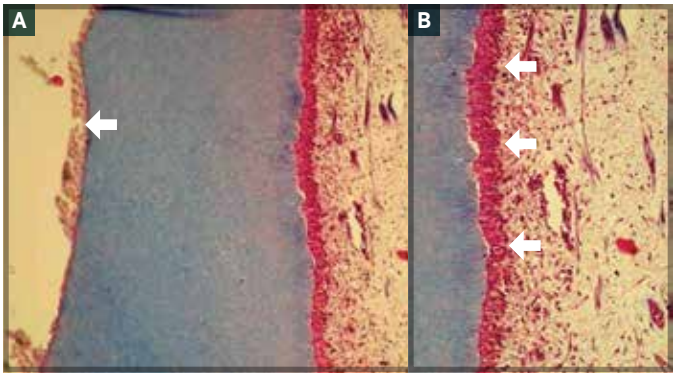

Fig 3. Tooth specimens from the VM group at 7 days. A. Relationship between the Class V cavity and the pulp. Note the presence of VM on the cavity floor (arrow). RDT, 0.524 mm (Masson trichrome; original magnification 54×). B. Detail of Fig 3A showing the pulp tissue at the cavity floor. A continuous layer of odontoblasts underlies the tubular dentin (arrows) (Masson trichrome; original magnification 125×).

At 30 days, 3 specimens exhibited no inflammatory pulpal response. In 1 specimen (RDT, 0.221 mm), a diffusion of VM components across dentinal tubules was observed; the subjacent pulp tissue presented disruption of the odontoblastic layer associated with moderate mononuclear inflammatory response and zones of inner dentin resorption (Fig 4). Despite the very thin RDT measured in this specimen, the sections stained with the Brown & Brenn technique did not show the presence of bacteria on the cavity walls or inside dentinal tubules. Bacteria were found in 1 specimen (RDT, 0.417 mm) in which no inflammatory response or tissue disorganization was observed. The mean value of the RDT for this experimental group was 0.381 mm.

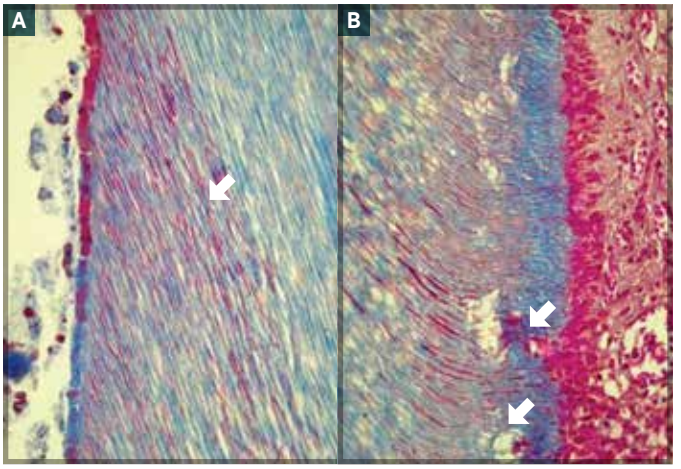

Fig 4. Tooth specimens from the VM group at 30 days. A. Cavity floor on which VM was applied as liner after etching of dentin. Note the intense inward diffusion of dental material components through the subjacent dentinal tubules (arrow). RDT, 0.221 mm (Masson trichrome; original magnification 250×). B. Detail of the pulp tissue at the cavity floor. A small amount of dental material components reaching the predentin can be observed. The subjacent pulp tissue exhibits disruption of odontoblasts and a persistent inflammatory reaction associated with resorption of inner dentin (arrows) (Masson trichrome; original magnification 250×).

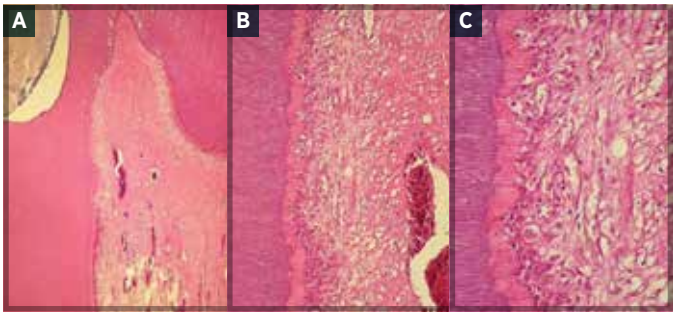

Fig 5. Tooth specimens from the Vitrebond (VB) group at 7 days. A. Relationship between the Class V cavity and the pulp. Hyaline alteration of the extracellular matrix can be observed in the pulp horn at the cavity floor. RDT, 0.249 mm (hematoxylin and eosin [H&E]; original magnification 32×). B. Detail of Fig 5A showing the disruption of the odontoblastic layer at the cavity floor and the mild inflammatory local pulp reaction (H&E; original magnification 64×). C. High magnification of Fig 5B showing the disrupted layer underlying continuous predentin. Numerous small blood vessels are present among mononuclear inflammatory cells (H&E; original magnification 125×).

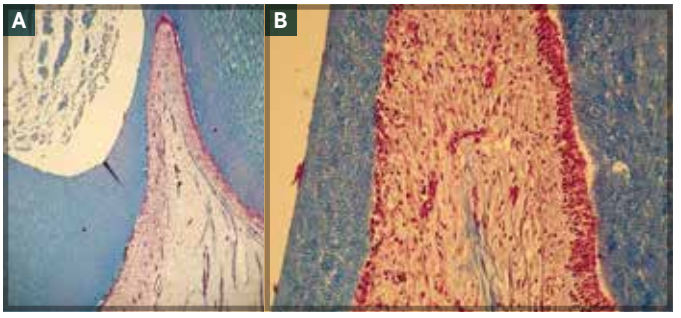

Fig 6. Tooth specimens from the VB group at 30 days. A. Relationship between the Class V cavity and the pulp. RDT, 0.199 mm (Masson trichrome; original magnification 32×). B. Detail of Fig 6A showing the cavity floor and the subjacent pulp tissue. Note the reduced number of odontoblasts at the cavity floor lined with VB. Discrete tissue disorganization associated with a slight inflammatory pulpal reaction is observed (Masson trichrome; original magnification 125×).

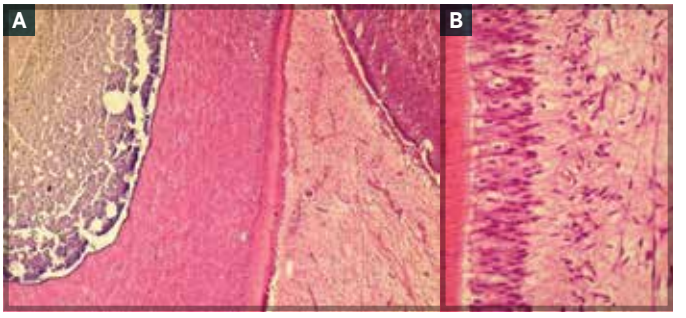

Fig 7. Tooth specimens from the VB group at 30 days. A. Relationship between Class V cavity and the pulp. A thin layer of reactionary dentin has been deposited by odontoblasts organized in a continuous layer. RDT, 0.451 mm (H&E; original magnification 86×). B. Detail of Fig 7A showing the pulp tissue at the cavity floor. Tubular reactionary dentin, a homogenous odontoblastic layer, a cell-free zone, and a subjacent cell-rich zone are observed (H&E; original magnification 280×).

VB group

At 7 days, 4 specimens (RDT, 0.249-0.351 mm) exhibited mild inflammatory responses mediated by mononuclear cells and the presence of a number of small, congested blood vessels. In the evaluation of all 6 specimens in this group, either no or discrete tissue disorganization was observed; disorganization that was present was characterized by disruption of the odontoblastic layer subjacent to the cavity floor (Fig 5). In 2 specimens (RDT, 0.512 mm and 0.471 mm), no inflammatory response or tissue disorganization was observed. Bacteria were not found in any of the sections stained with the Brown & Brenn technique. "No tertiary dentin deposition" was a common histologic finding for all specimens evaluated. The mean RDT for this experimental group was 0.369 mm.

At 30 days, no inflammatory response or tissue disorganization was observed in 5 specimens. Only 1 specimen (RDT, 0.199 mm) exhibited reduced numbers of odontoblasts in the pulp zone subjacent to the cavity floor. In this specimen, a discrete inflammatory reaction and tissue disorganization were observed (Fig 6). Bacteria were not found in any of the sections stained with the Brown & Brenn technique. Discrete reactionary dentin deposition was observed in 1 specimen with an RDT of 0.451 mm (Fig 7). The mean RDT for this experimental group was 0.373 mm.

DY group

At the 7-day (n = 6) and 30-day (n = 4) periods, no specimens exhibited an inflammatory response or tissue disorganization. Bacteria at the lateral walls of the dental cavity were evident in only 1 specimen at 7 days (RDT, 0.495 mm). However, in this specimen, as well as in all the other specimens of this group, the odontoblastic layer at the cavity floor was continuous with that surrounding the coronal pulpal space, and cell-free and cell-rich zones were preserved. The mean RDTs for the specimens evaluated at 7 and 30 days were 0.400 mm and 0.335 mm, respectively.

Intact group

The normal histologic pulp features observed in the DY control group also occurred in the intact control group, in which sound teeth were used to confirm the quality of the histologic processing (Fig 8).

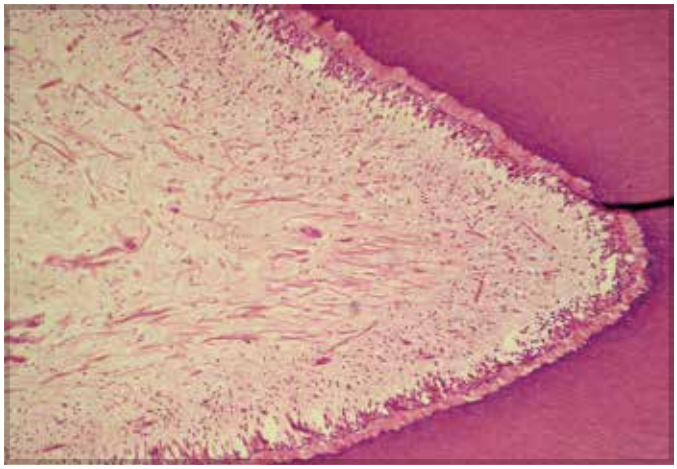

Fig 8. Tooth specimen from the intact control group showing a general view of the pulp tissue of a sound tooth. The pulp horn exhibits normal histologic characteristics (H&E; original magnification 64×).

Discussion

The potential toxicity of RMGICs has been mainly related to the release of HEMA when these materials are applied in wet environments.[15,16] Due to the low molecular weight and high hydrophilicity of HEMA, this monomer may easily diffuse through dentinal tubules to cause toxic effects to different cell lineages, such as lymphocytes, monocytes, macrophages, osteoblasts, and human dental pulp cells.[17-28] Direct contact of HEMA with cells inhibits cell growth, arrests the cell cycle, and promotes apoptosis, all of which are associated with glutathione content depletion and oxidative stress generation.[29-34]

However, although HEMA is part of the chemical composition of RMGICs, these resin-based cements have been indicated to line or restore deep cavities prepared in vital teeth.[8,31] Costa et al demonstrated that VB caused no significant alteration to the pulp of human premolars, even when the material was applied in cavities with an RDT less than 0.3 mm.[7] However, different formulations and application protocols of RMGICs, which may directly affect the biocompatibility of these materials, have been widely used without prior evidence of safety.[4,8,9]

Vitremer, which contains a high concentration of HEMA, presents improved mechanical properties compared with VB and has been widely indicated for cavity restoration.[8,9,31] According to the manufacturer, no application of a biocompatible liner is required before the restoration of dental cavities with VM.[12] However, the application protocol of VM includes the pretreatment of dentin substrate with a light-cured acidic primer containing HEMA. No previous in vivo studies in young human permanent teeth have been performed to assess the biocompatibility of VM applied to dentin regardless of cavity depth, so the authors of the present investigation compared this RMGIC to the liner/base VB and the hard-setting DY.

In the present study, the pulp tissue of premolars lined with DY exhibited normal histologic characteristics, as shown in previous studies.[7,10] For this reason, CHC has been widely used by researchers as the gold standard to assess and compare the biocompatibility of new dental materials indicated to be clinically used as liners/bases.[7,10] However, hard-setting CHCs are highly soluble in wet environments and present poor mechanical properties.[32,33] In addition, CHCs do not adhere to dentin substrate or resin-based restorative materials. Consequently, in spite of the antibacterial activity and biocompatibility of CHCs, they do not appear to be the best material for application to the tubular dentin of the highly permeable cavity floor.[32]

As odontoblasts form a monolayer at the periphery of dentin, and their protracted processes are anchored into dentinal tubules, these cells are the first to be sensitized by a chemical challenge from dental materials.[34,35] The disruption of the odontoblastic layer is one of the first histologic features to indicate that pulpal homeostasis has been disturbed. It has been reported that odontoblasts can elicit an inflammatory pulpal reaction, since they are able to synthesize proinflammatory cytokines and chemokines, initiating the defense mechanisms of pulp tissue.[34-36] In the present study, the slight inflammatory pulpal reaction and tissue disorganization observed 7 days after the cavity floor was lined with VB were resolved at the 30-day period. As shown by Costa et al, these histologic events indicate the biocompatibility of this resin-based cement and confirm the reparative capacity of pulp tissue, even when VB is applied as a liner in very deep cavities prepared in human teeth.[7]

At the 7-day period, VM also caused a slight inflammatory pulpal response after being applied as a liner in cavities in which the RDT was 0.3-0.5 mm. However, greater alterations in the pulp tissue occurred in those teeth in which the cavities had an RDT less than 0.3 mm. In these specific teeth, the complete disruption of the odontoblastic layer as well as the inner dentin resorption persisted until 30 days after cavity restoration.

A similar pulpal response was observed in a 2002 study in which very deep dentin substrate of dental cavities prepared in human teeth was conditioned with acidic solutions and sealed with bonding agents.[10] The researchers found that the inward dentinal diffusion of toxic components leached from resinous materials applied to wet, highly permeable dentin caused pulpal damage.[10] The authors reported that the unreacted toxic monomers that reached the pulp may have also inhibited local odontoblastic secretory activity and the odontogenic differentiation capability of mesenchymal stem cells, resulting in no deposition of tertiary dentin, a finding that was in agreement with the results of the present investigation. In vitro studies have

demonstrated that HEMA and other resin monomers inhibit the odontoblastic differentiation of dental pulp stem cells, as indicated by the inhibition of type 1 collagen, osteonectin, and dentin sialoprotein messenger RNA expression as well as the absence of mineralized nodule deposition.[23,37,38] In addition, pulp cells exposed to HEMA presented an apparent retraction and loss of filopodia and lamellipodia, the extended cellular processes that are crucial for cell proliferation and movement during wound healing and tissue morphogenesis.[24,27]

A previous study demonstrated that VM presents less water sorption and solubility, better mechanical properties, and faster setting reaction than VB.[39] These positive properties of VM have been related to the greater amount of HEMA in its composition as well as to its higher powder-liquid ratio.[31,39,40] Therefore, it seems that liner/base dental materials such as VB, when immersed in water, absorb greater amounts of water and release a greater quantity of HEMA than do restorative RMGICs.[15,16] This may explain why the eluates from VB were intensely more cytotoxic to cultured odontoblast-like cells than those obtained from VM, and why VB caused greater inflammatory reactions than VM when both materials were implanted in the subcutaneous connective tissue of rats.[16]

While the methodologies of cytotoxicity on cell culture and connective tissue implantation are both recommended by the International Organization for Standardization as means of materials testing, the scientific data obtained from these types of studies cannot be directly extrapolated to clinical situations.[3] In the present in vivo investigation, VM applied to very deep cavities (RDT < 0.3 mm) prepared in human premolars, following the protocol recommended by the manufacturer, triggered a more intense pulpal reaction than VB. This suggests that, rather than the HEMA present in VM, it was the pretreatment of dentin with an acidic resin-based light-cured primer—as recommended by the manufacturer in cases of cavity restoration—that played the major role in the pulpal damage observed microscopically.[12] This concurs with the findings of About et al, who reported that both VB and VM presented the same aggressive potential to pulp tissue when applied to dentin pretreated with 37% phosphoric acid.[41]

Vitremer primer is composed of polycarboxylic acid, ethanol, photoinitiators, and a high concentration (46%) of HEMA.[12] According to Di Nicoló et al, treatment of dentin with this primer allows a more effective bond to this tubular substrate.[42] This procedure improves HEMA penetration into the dentinal tubules, creating micromechanical retention as well as chemical adhesion to the dentin.[43,44] Since the mechanism of RMGIC adhesion to dentin is based on ionic bond formation between carboxyl groups of polyalkenoic acid and calcium hydroxyapatite as well as micromechanical retention, the smear layer restrains effective contact between the restorative material and the subjacent dentin substrate, impairing satisfactory adhesion.[44] However, acidic conditioning of very deep dentin allows a more intense inward diffusion of resin components into the dentinal tubules.[17] When very deep cavities prepared in human premolars were conditioned with a 35% phosphoric acid solution for 15 seconds before the application of a bonding agent, an intense inflammatory reaction was observed in the pulp tissue.[10] In contrast, no pulpal damage occurred after the same bonding agent was applied to unconditioned dentin.[10]

Di Nicoló et al reported that the pH of VM primer is not low enough to remove the smear layer completely from the dentin surface, thus allowing an amorphous structure to remain that covers the dentinal tubules, preventing formation of an RMGIC-dentin interdiffusion zone.[42] However, it seems that partial smear layer removal from the floor of very deep cavities does not prevent the inward diffusion of toxic products capable of causing pulpal damage, as observed in the present study. It is likely that the HEMA present in the VM primer diffused through the dentinal tubules during its application to dentin. Based on the fact that the light-curing procedure promotes inward dentinal fluid movement, free toxic HEMA from the VM primer may have easily diffused through the dentinal tubules to elicit a chronic inflammatory pulpal reaction followed by resorption of inner dentin.[1]

Some clinical studies have reported no pulpal alterations when VM was applied to cavities pretreated with acidic agents.[45,46] Marchi et al used VM to restore very deep cavities prepared in primary molars after pretreating the dentin surface with a 10% phosphoric acid solution and applying VM primer.[45] At a 4-year follow-up, no clinical or radiographic signs or symptoms of pulpal alterations were recorded for most of the restored teeth. Falster et al also used deep cavities prepared in primary molars.[46] They performed a clinical and radiographic evaluation of indirect pulp capping using a CHC or a total-etch adhesive system and reported 83% and 96% success rates for the CHC and adhesive systems, respectively, in an analysis at 2-year follow-up. The authors concluded that the success of indirect pulp capping does not depend on the dental material applied to dentin.[46]

However, analysis of the data obtained from the aforementioned clinical trials contradicts the results of the present investigation and other in vivo studies in which histopathologic evaluations of pulp tissue were performed.[7,10] It is important to note that, in studies performed in human teeth, microscopic findings had no direct correlation with clinical observations, since patients reported no discomfort during experiments, and normal radiographic images were observed even when an intense inflammatory response was present in the pulp tissue.[7,10] According to a recent study, clinical and radiographic evidence cannot support the introduction of a new therapy in dentistry, and only the histopathologic analysis of pulp tissue can determine the biocompatibility of new dental materials.[3] Therefore, the histologic features of the pulp tissue observed in the present study confirm that VB and DY are biocompatible when applied to very deep cavities. However, pretreatment of dentin with an acidic primer containing HEMA prior to the application of VM to cavities with an RDT less than 0.3 mm resulted in prolonged injury to pulp tissue, similar to that observed when bonding agents were used to seal very deep cavities pretreated with phosphoric acid.[10] The original data obtained from teeth in which very deep cavities were lined with VM determined the rejection of the null hypothesis of the present in vivo study.

Conclusion

Based on the methodology used in the present in vivo study, it can be concluded that VB and DY are biocompatible when applied to very deep cavities prepared in permanent human teeth with an RDT less than 0.3 mm. In contrast, VM cannot be considered biocompatible, since the application of this material to dental cavities pretreated with an acidic light-cured primer containing HEMA caused persistent damage to the subjacent pulp tissue.

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