

# TISSUE REACTIONS INDUCED BY DENTAL PULP CAPPING MATERIALS

*Alexandru Sitaru, University Assistant*

*Tudor Hantoiu, University Assistant*

*Monica Monea, Associate Prof., PhD*

University of Medicine and Pharmacy Targu Mures, Romania

---

## Abstract

*Objective:* The aim of this article is to evaluate the biocompatibility of materials used in pulp capping procedures, by measuring the degree of inflammation induced by these products after subcutaneous implantation in rats. *Materials and methods:* we used 12 animals divided in 3 study groups and 1 control group; implants with Mineral trioxide Aggregate (MTA), Life cement (Kerr) and Calxyd (Spofa Dental) were placed in subcutaneous tissue and histological evaluation was carried out after 7, 14 and 21 days. *Results:* We obtained comparable results between MTA and Life cement, which demonstrates that these materials have similar degrees of biocompatibility. *Conclusion:* Based on this experimental study, MTA and calcium hydroxide containing cements express similar biocompatibilities, but this is not enough in order to eliminate the letter from every day dental practice. Further clinical studies are necessary in order to evaluate the potential of MTA to induce dentin bridge formation on exposed dental pulp.

---

**Keywords:** MTA, biocompatibility, dental pulp

## Introduction

Biocompatibility is a condition that must characterize all materials used in conservative dentistry, which means no risk of side effects on contact with host tissues (Modena da Silva et al., 2009; Scott et al., 2004). In the case of pulp capping materials, this is expressed by many variables such as genotoxicity, mutagenicity, carcinogenesis, cytotoxicity, histocompatibility and antimicrobial effect (Kleinsasser et al., 2004; Auschill et al., 2003).

Calcium hydroxide  $\text{Ca}(\text{OH})_2$  was the most used material in conservative dentistry, which in contact with vital pulp tissues determines a small area of necrosis accompanied by a mild inflammatory reaction (Bogen et al., 2008). In the absence of microorganisms, reparative reactions take place and a dentin bridge is formed underneath the  $\text{Ca}(\text{OH})_2$ . Unfortunately,

this material has a marked tendency to dissolve in time, leaving empty spaces, which represent opportunities for bacterial infiltration.

Taking into consideration that  $\text{Ca}(\text{OH})_2$  cements induce the development of dentin bridges with tunnel-like defects, that allow bacterial penetration from the oral cavity to the pulp tissue, other materials were introduced with less marginal leakage and better isolation of the pulp chamber. Over the years, researchers used zinc oxide eugenol, glass ionomer cements, dentinal adhesive systems and, recently, a new material called “Mineral Trioxide Aggregate” (MTA). Due to its alkaline pH, it was shown to stimulate dentin bridge formation, with very good results in direct pulp capping procedures (Accorinte et al., 2005).

The purpose of our study was to evaluate the biocompatibility of frequently used materials in conservative treatment of vital pulp, such as MTA and two  $\text{Ca}(\text{OH})_2$  containing products: Life cement and Calxyd paste. We measured the degree of inflammation induced after subcutaneous implantation of these materials in experimental animals.

### **Main Text:**

According to international laws, our test was conducted respecting the criteria of ISO 10.993 and ISO/Tc 194; we obtained the acceptance No. 58/2011 from Committee for Ethics of Research of the University of Medicine and Pharmacy of Tg. Mureş. As bio-test, we used a species of laboratory rats and the materials included in this study were implanted in subcutaneous tissue. There were 4 groups consisting of 12 animals, 3 study groups and 1 control. Each study group received implants of sterile cotton with MTA, Life cement (Kerr) and Calxyd (Spofa Dental) respectively, and in the control group only sterile cotton pellets were implanted.

The procedure was carried out by a single operator, who used local anesthesia with 2 ml Mebumal 10%, following general rules of asepsis and antisepsis; postoperatively, the animals were not isolated and no antibiotherapy was used, in order to avoid any effect on tissue inflammatory reactions. Under the same protocol, specimens of connective tissue were obtained at 7, 14 and 21 days. All fragments were immediately introduced in formaline solution and prepared for histological evaluation. We used hematoxylin-eosin and/or PAS stains and the evaluation was carried out by one examiner, in a double-blind manner.

The degree of inflammation was evaluated in 4 stages, based on the criteria of Commission of Dental materials, instruments, equipment and techniques (1980) in the following manner:

0 – absent: width of inflammatory zone similar to control group, absence of or only a few inflammatory cells (no more than 5 cells);

1 – moderate: mild inflammatory reaction, macrophages and plasma cells (5-25 cells);

2 – intense: very strong inflammatory reaction, macrophages, plasma cells, with foci of granulocytes and lymphocytes (25-100cells);

3 – severe: areas of necrosis, numerous inflammatory cells in the surrounding tissue (more than 125 cells).

Quantitative assessment of inflammatory cells was carried out in 5 separate fields of each specimen, the mean count was determined and the severity of inflammatory response was noted. Data from the experimental groups (MTA, Life cement and Calxyd) and control group was compared using Friedman statistical test for each period of time (7, 14 and 21 days). Wilcoxon complementary test was used to determined differences between study groups ( $p < 0,05$ ).

## Results

The severity of tissue inflammatory response for all these dental pulp capping materials was high. The most severe reaction was noted in the case of Calxyd, followed by MTA, Life and control groups. Cellular distribution scores for the experimental and control groups and the corresponding tissue reactions are presented in Tabel 1. Overall, the inflammatory cell infiltrate decreased from day 7 to day 21 in all groups and the development of a collagen membrane around the implant increased during the study period. A fibrous capsule formation was completed in the control group by day 21.

**Table 1.** Cellular distribution scores in all groups for each time interval

Cell type	Time	7 days				14 days				21 days			
	Study group	A	B	C	D	A	B	C	D	A	B	C	D
<i>PMN Leucocytes</i>		3	3	3	2	2	2	1	0	1	1	1	0
<i>Lymphocyt</i> s		3	3	2	2	2	2	1	1	1	1	0	0
<i>Macrophages</i>		2	1	2	1	1	2	1	0	1	0	0	0
<i>Giant cells</i>		2	1	1	1	1	1	0	0	0	0	0	0
<i>Necrosis</i>		+	+	+	+	+	+	-	-	-	-	-	-
<i>Fibrous capsule</i>		-	-	-	-	-	+	+	+	+	+	+	+

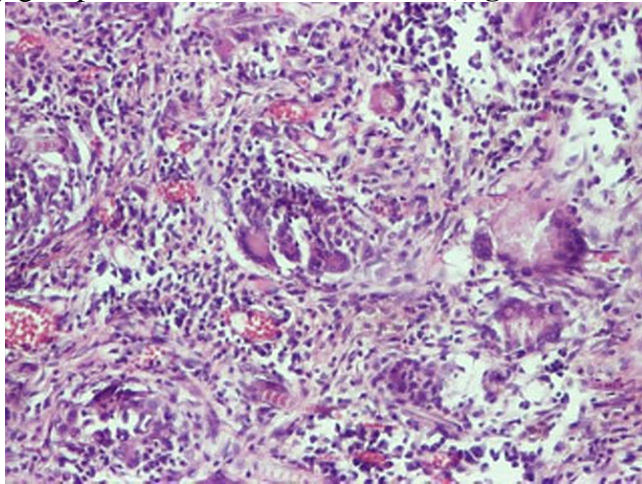
After 7 days, the Friedman test indicated a significant difference between the groups ( $p < 0,05$ ) with Calxyd paste showing the most severe response. On the other hand, the Wilcoxon complementary test did not show significant differences between Life cement and MTA.

After 14 and 21 days, the Friedman test indicated significant differences between the groups ( $p < 0,05$ ), but with the Wilcoxon complementary test there were no differences between Calxyd – control, MTA – control and Life – control groups. The sealer groups did not show any significant differences among them (Tabel 2).

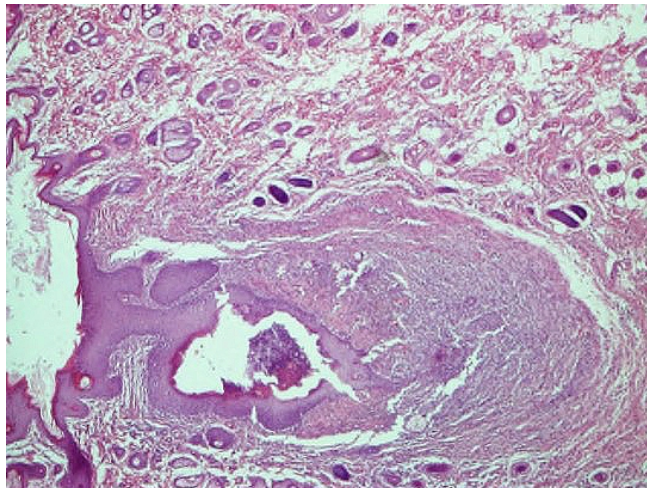
**Table 2.** P values regarding the intensity of inflammatory reactions induced by different experimental materials

<i>Group</i> <i>Time</i>	<i>Calxyd</i>	<i>MTA</i>	<i>Life</i>	<i>Control</i>
7 days	0.275	0.317	0.163	0.011
14 days	0.502	0.448	0.316	0.038
21 days	0.319	0.229	0.173	0.014

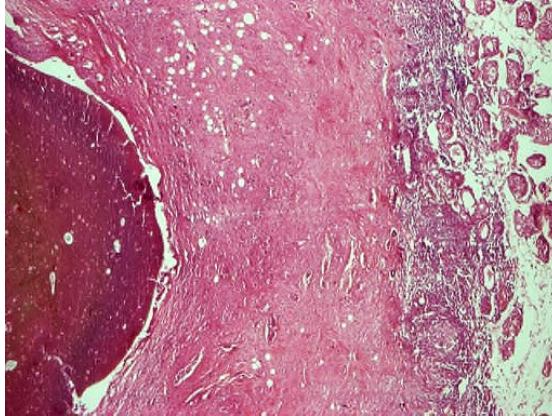
In the following pictures, we present the histological aspects obtained from all study groups at different time intervals (**Figure 1- 9**):



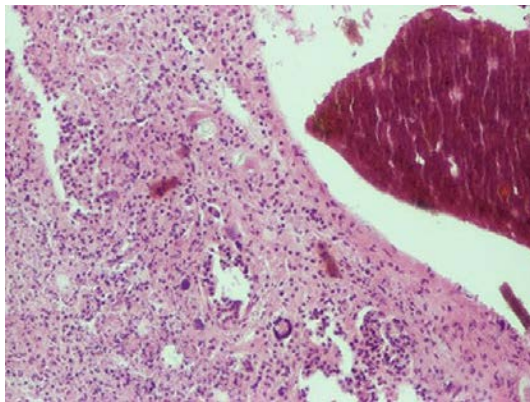
**Fig.1** Control group at 7 days. Moderate inflammatory infiltrate, multinucleated cells, enlarged blood vessels (H&E stain, 20X).



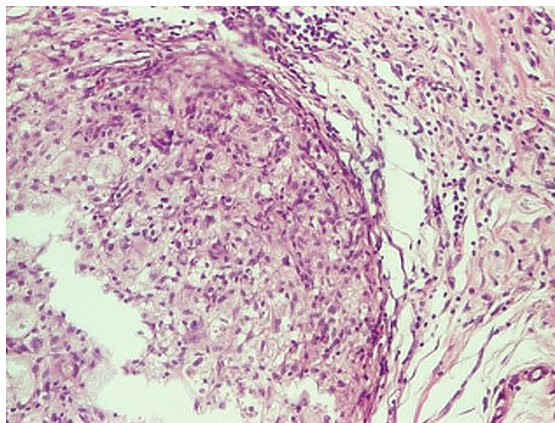
**Fig.2** MTA at 7 days. The specimen displays a moderate inflammation, with a severe inflammatory infiltrate. The implant placed in epithelial tissue is surrounded by a limited necrotic zone. (H&E stain, 20X).



**Fig.3** Calxyd paste at 7 days. Large necrotic zone, severe inflammatory infiltrate, numerous hyperemic blood vessels. (H&E stain, 20X)

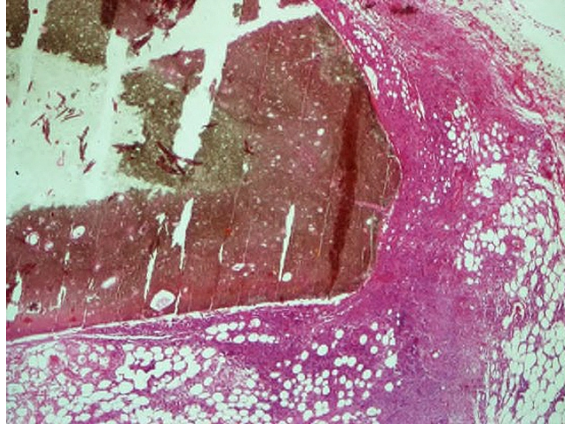


**Fig.4** Life cement at 14 days. Connective tissue barrier is developing around the implant, the inflammatory infiltrate is moderate. There are numerous collagen fibres and fibroblasts which express tissue healing reactions. (H&E stain, 20X).

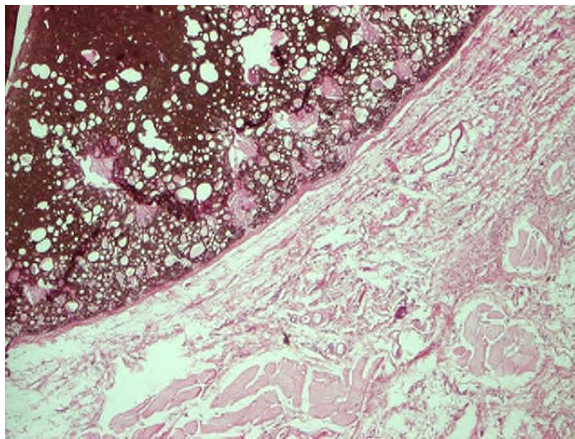


**Fig.5** MTA at 14 days. Slight tendency to tissue healing and reduction of the inflammatory infiltrate. Connective tissue barrier was formed, there are numerous collagen fibres and a few macrophages. (H&E stain, 20X).

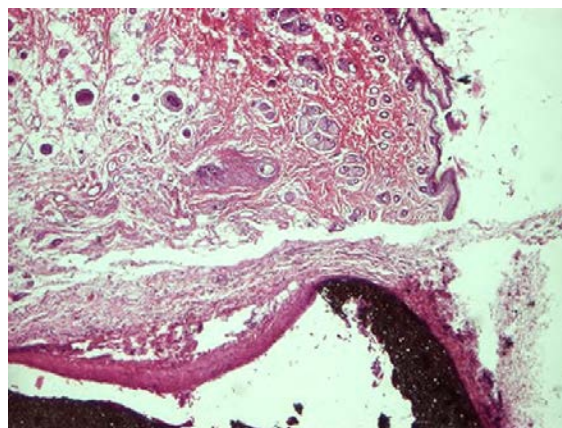




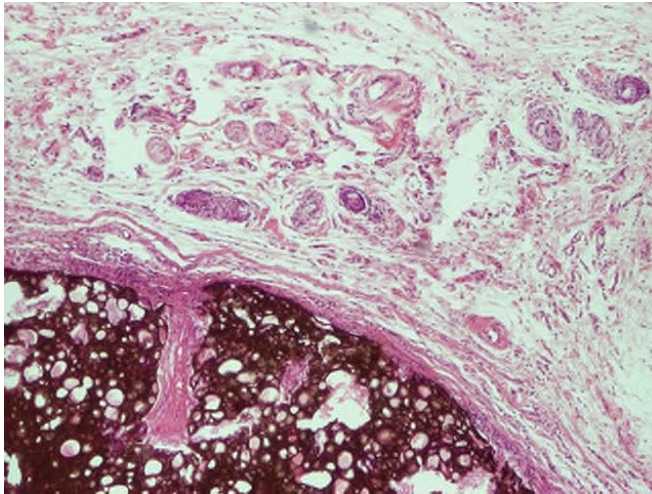
**Fig.6** Control at 21 days. The implant placed in subcutaneous tissue shows healing with development of a fibrous barrier. Absence of inflammatory cells. (H&E stain, 10X).



**Fig.7** MTA at 21 days. Tendency to tissue healing and absence of the inflammatory infiltrate. The implant was rarefied due to macrophages activity and a thin fibrous capsule is developed. (PAS stain, 20X).



**Fig.8** Calxyde paste at 21 days. The implant is surrounded by a fibrous capsule, there is no inflammation and the tissue is considered healed . (H&E stain, 10X).



**Fig. 9.** Life cement at 21 days. The thick fibrous barrier with a septum surrounds the implant and the adjacent tissue is healed. (H&E stain, 20X).

## Discussion

Conservative treatment of the dental pulp is still a subject of intense debate, as there are numerous methods that totally or partially preserve the vitality of this tissue. The success rate is strongly correlated with the age of the patient, type of exposure, period of time elapsed until treatment, absence of inflammation and infection being very important positive prognostic factors (Szep et al., 2002; Bouillaguet et al., 2002; Pelka et al., 2000; Tai et al., 2002)

Direct pulp capping was the method of choice in young patients with traumatic pulp exposure who come for treatment during the first 24 hours. In these conditions, the correct control of hemorrhage, isolation and coverage of the defect with a biocompatible material under a tight seal offers best condition for healing by new dentin bridge formation. Therefore, the ideal pulp capping material has to adhere strongly to dental hard tissues, prevent microleakage, be insoluble in oral fluids, have a bactericidal effect and, finally yet importantly, be biocompatible (Zmener et al., 2004).

The most used material for pulp capping procedures is calcium hydroxide, which has a strong antibacterial effect and stimulates neodentinogenesis. In direct contact with vital tissue, it will determine a limited zone of necrosis, due to a pH of 11-12 in freshly mixed state. The healing process will start under this scar only if there is no microbial infiltration from the oral cavity (Aranha et al., 2006; Saw et al., 2005; De Souza et al., 2007). Recently, many clinical studies presented very good results after pulp capping of immature teeth with MTA, which is now considered superior to  $\text{Ca(OH)}_2$  regarding biocompatibility and hard tissue formation (Parirokh et al., 2010).

MTA has an initial pH of 10.2, which rises to 12.5 after 3 hours; applied on dental pulp it induces the release of inflammatory cytokines and development of a hard barrier that resembles hydroxyapatite (Torabinejad et al., 2010; Tuna et al., 2008). Therefore, one can say that MTA induces cellular and functional mechanisms at the surface of pulp tissue, offering an active biologic substrate with strong neodentinogenetic effect (Whiterspoon, 2008; Matt et al., 2004; Tselnik et al., 2004).

Our investigation was based on an experimental method widely used in *in vivo* studies and is currently considered the most accurate approach in evaluating the degree of inflammation induced by different dental materials used in modern dentistry.

Regarding the effect of surgical procedure upon experimental animals, we noticed no difficulty in the placement of subcutaneous samples, which was easily done by a skin incision, and the general health and behavior was not affected by the presence of these implants. After 7 days, the histological examination revealed the development of inflammatory reactions at the control group and the study groups with Life cement, MTA and Calxyd paste, ranked as intense (2), and severe (3), respectively.

At 14 days postoperatively, the degree of inflammation showed clear signs of reduction; we noticed tissue healing and therefore all samples from study groups were ranked 1, corresponding to moderate inflammation. The common feature of tissue reactions were a clear demarcation of an inflammatory zone, surrounded by a fibrous capsule with many fibroblasts, which is considered a normal response reaction to subcutaneous implants. There was also a localized inflammatory infiltrate.

After 21 days, the clinical examination revealed a scar tissue, which exerted no pain on palpation. The surgical exposure of the implant showed that it became attached to the skin by a fibro-conjunctive transparent capsule, with a mild congestive reaction.

## **Conclusion**

Subcutaneous implants of dental pulp capping materials such as MTA, Life cement, Calxyd by a surgical procedure do not determine alterations of general state of health of the experimental animals.

The inflammatory tissue reactions observed after implantation of MTA and Life cement are comparable, demonstrating that there are no significant differences in biocompatibility of these materials.

Anyway, this is not enough to rule out calcium hydroxide from every day dental practice, due to its excellent results.

Further clinical studies are necessary in order to evaluate the potential of MTA to induce dentin bridge formation on exposed dental pulp and its long-term clinical outcome.



## References:

- Modena da Silva KC, Casas-Apayco LC, Atta MT, Costa CA, Hebling J, Sipert CR, Navarro M, Santos CF. *Cytotoxicity and biocompatibility of direct and indirect pulp capping materials*. J Appl Oral Sci. 2009; 17(6): 544-554
- Scott, A.; Egner, W.; Gawkrödger, D.J.; Hatton, P.V.; Sherriff, M.; van Noort, R.; Yeoman, C.; Grummitt, J. *The national survey of adverse reactions to dental materials in the UK: a preliminary study by the UK Adverse Reactions Reporting Project*. Br. Dent. J. 2004, 196, 471-477.
- Kleinsasser, N.H.; Wallner, B.C.; Harreus, U.A.; Kleinjung, T.; Folwaczny, M.; Hickel, R.; Kehe, K.; Reichl, F.X. *Genotoxicity and cytotoxicity of dental materials in human lymphocytes as assessed by the single cell microgel electrophoresis (comet) assay*. J. Dent. 2004, 32, 229-234.
- Auschill TM, Arweiler NB, Hellwig E, Sculean A. *Success rate of direct pulp capping with calcium hydroxide*. Schweiz Monatsschr Zahnmed 2003; 113(9): 946-952.
- Bogen G, Kim JS, Bakland LK. *Direct pulp capping with Mineral Trioxide Aggregate: An observational study*. J Am Dent Assoc, 2008; 139; 305-315.
- Accorinte Rodriguez ML, Lognercio AD, Reis A, Muench A, Cavalcanti de Aranjó V. *Adverse effects of human pulps after direct pulp capping with different components from a total-etch three step adhesive system*. Dental Materials, 2005, 21: 599-607.
- Szep, S.; Kunkel, A.; Ronge, K.; Heidemann, D. *Cytotoxicity of modern dentin adhesives—in vitro testing on gingival fibroblasts*. J. Biomed. Mater. Res. 2002, 63, 53-60.
- Bouillaguet, S.; Shaw, L.; Gonzalez, L.; Wataha, J.C.; Krejci, I. *Long-term cytotoxicity of resin based dental restorative materials*. J. Oral Rehabil. 2002, 29, 7-13.
- Pelka, M.; Danzl, C.; Distler, W.; Petschelt, A. *A new screening test for toxicity testing of dental materials*. J. Dent. 2000, 28, 341-345.
- Tai, K.W.; Huang, F.M.; Huang, M.S.; Chang, Y.C. *Assessment of the genotoxicity of resin and zinc-oxide eugenol-based root canal sealers using an in vitro mammalian test system*. J. Biomed. Mater. Res. 2002, 59, 73-77.
- Zmerner, O. *Tissue response to a new methacrylate-based root canal sealer: preliminary observations in the subcutaneous connective tissue of rats*. J. Endod. 2004, 30, 348-351.
- Aranha, A.M.; Giro, E.M.; Souza, P.P.; Hebling, J.; de Souza Costa, C.A. *Effect of curing regime on the cytotoxicity of resin-modified glass-ionomer lining cements applied to an odontoblast-cell line*. Dent. Mater. 2006, 22, 864-869.

- Saw, T.Y.; Cao, T.; Yap, A.U.; Lee Ng, M.M. *Tooth slice organ culture and established cell line culture models for cytotoxicity assessment of dental materials*. *Toxicol. In Vitro* 2005, 19, 145-154.
- de Souza Costa, C.A.; Teixeira, H.M.; Lopes do Nascimento, A.B.; Hebling, J. *Biocompatibility of resin-based dental materials applied as liners in deep cavities prepared in human teeth*. *J. Biomed. Mater. Res. Appl. Biomater.* 2007, 81, 17
- Parirokh M, Torabinejad M. *Mineral trioxide aggregate, a comprehensive literature review: part I. Chemical, physical and antibacterial properties*. *J Endod* 2010, 36: 16-27.
- Torabinejad M, Parirokh M. *Mineral trioxide aggregate: a comprehensive literature and review: part II. Leakage and biocompatibility investigations*. *J Endod* 2010, 36: 190-202.
- Tuna D, Olmez A. *Clinical long-term evaluation of MTA as a direct pulp capping material in primary teeth*. *Int Endod J.* 2008, 41(4): 273-278.
- Whiterspoon DE. *Vital pulp therapy with new materials. New directions and treatment perspectives in permanent teeth*. 2008, 34: 525-528.
- Matt GD, Thorpe JR, Strother JM, McClanahan SB. *Comparative study of white and gray mineral trioxide aggregate (MTA) simulating a one-or two-step apical barrier technique*. *J Endod* 2004;30:876–9.
- Tselnik M, Baumgartner JC, Marshall JG. *Bacterial leakage with mineral trioxide aggregate or a resin-modified glass-ionomer used as a coronal barrier*. *J Endod* 2004;30:782–4.