International Endodontic Journal

doi:10.1111/j.1365-2591.2011.01874.x

Immunohistochemical analysis of subcutaneous tissue reactions to methacrylate resin-based root canal sealers

Y. Yamanaka, Y. Shigetani, K. Yoshiba, N. Yoshiba & T. Okiji

Division of Cariology, Operative Dentistry and Endodontics, Department of Oral Health Science, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

Abstract

Yamanaka Y, Shigetani Y, Yoshiba K, Yoshiba N, Okiji T. Immunohistochemical analysis of subcutaneous tissue reactions to methacrylate resin-based root canal sealers. *International Endodontic Journal*, **44**, 669–675, 2011.

Aim To investigate subcutaneous tissue reactions to methacrylate resin-based root canal sealers by immunohistochemical assessment of inflammatory/immunocompetent cell infiltration.

Methodology Silicone tubes containing freshly mixed Epiphany SE sealer, MetaSEAL, Super-Bond RC sealer, or a zinc oxide-eugenol sealer (Canals) were subcutaneously implanted into the backs of Wistar rats. Solid silicone rods implanted in different animals served as controls. After 7, 14 and 28 days, connective tissue surrounding the implants ($n = 8$, each) was processed for immunoperoxidase staining using OX6 (reactive to major histocompatibility complex class II molecules), ED1 (reactive to macrophages), and W3/13 (reactive primarily to neutrophils), and the number of positively stained cells within each field (1.2×0.8 mm) was enumerated. Statistical differences were analysed with Friedman's test and Scheffe's test (comparisons between test materials) or Mann–Whitney's *U*-test (test–control comparisons).

Results Canals showed a significantly higher number of W3/13-positive cells (mostly neutrophils) than MetaSEAL at 28 days ($P < 0.05$). There were no significant differences in the numbers of OX6- or ED1-positive cells between each test material at any time point. Test–control comparisons revealed several significant differences for each antibody. This was most notable for ED1, where all the test materials at each time point, except for Epiphany SE at 28 days, showed significantly larger values than the corresponding controls.

Conclusions All the methacrylate resin-based sealers tested showed a similar level of inflammatory/immunocompetent cell infiltration. MetaSEAL induced less-intense neutrophil infiltration than Canals. Controls exhibited milder infiltration of inflammatory/immunocompetent cells compared with all the test materials.

Keywords: immunohistochemistry, methacrylate resin-based root canal sealers, subcutaneous tissue reaction.

Received 11 August 2010; accepted 5 February 2011

Introduction

Successful root canal treatment depends on the elimination of intracanal infection, followed by effective and biocompatible canal filling in order to avoid reinfection and irritation of the periradicular tissue (Sundqvist & Figdor 1998, Siqueira 2001). Root canal sealers are expected to be confined within the root canal (Ricucci & Langeland 1998, Bernáth & Szabó 2003); however, they may inadvertently extrude into the periradicular tissue and consequently cause tissue irritation and delayed healing (Seltzer 1999, Sari & Duruturk 2007). Thus, one of the principal requirements of an endodontic root canal sealer is that it is immunologically compatible with the periradicular tissue (Geurtsen & Leyhausen 1997). Antimicrobial activity is another

Correspondence: Yusuke Yamanaka, Division of Cariology, Operative Dentistry and Endodontics, Department of Oral Health Science, Niigata University Graduate School of Medical and Dental Sciences, 2-5274, Gakkocho-dori, Chuo-ku, Niigata city, 951-8514, Japan (Tel./fax: +81 25 227 2865; e-mail: y-yamanaka@dent.niigata-u.ac.jp).

© 2011 International Endodontic Journal

desirable property of a root canal sealer, although it should be considered that there may be a balance between antimicrobial activity and host cytotoxicity.

The recent advancement of dentine bonding technology has led to the development of several brands of methacrylate resin-based root canal sealers, which take advantage of the adhesive properties of methacrylate in an attempt to reduce leakage and strengthen the root (Schwartz 2006). Self-adhesive dual-curable sealers, which eliminate the need for a separate priming step, are the most recent generation of these materials and include Epiphany SE (a modified version of Epiphany, a nonetching methacrylate resin-based sealer), MetaSEAL (4-methacryloxyethyl trimellitic anhydride (4-META) containing methacrylate resin-based sealer), and the Super-Bond RC sealer, which is a 4-META/methyl methacrylate-tri-n-butyl borane (MMA-TBB) resin-based liquid-and-powder type sealer that was recently developed by modification of the Super-Bond resin cement.

However, the biological properties of methacrylate resin-based sealers have not yet fully been elucidated. In particular, only a few studies have examined self-adhesive sealers: *in vitro* studies have been demonstrated that they have relatively low cytotoxicity (Pinna *et al.* 2008, Ames *et al.* 2009, Gambarini *et al.* 2009); however, *in vivo* studies are lacking. The available information about the tissue compatibility of Super-Bond RC sealer is also limited, although Super-Bond resin cement seems to be reasonably biocompatible (Inoue *et al.* 2001, Fujisawa & Atsumi 2004, Yoshimine *et al.* 2007); studies have demonstrated that the Super-Bond RC sealer shows better cytocompatibility than other resin-based sealers (Eldeniz *et al.* 2007) and causes milder tissue reactions than a zinc oxide-eugenol sealer (Hemmi *et al.* 2003). Moreover, some components of methacrylate resin-based sealers may have inflammatory/immunogenic potential; 2-hydroxyethyl methacrylate (HEMA), a constituent in some methacrylate resin-based sealers (see Table 1), has been reported to possess inflammatory and adjuvant properties (Sandberg *et al.* 2005). However, little information is available regarding the immunogenicity of different resin-based sealers.

Subcutaneous implantation is widely used to assess the *in vivo* tissue reaction of root canal sealers (Görduysus *et al.* 1998, Zmener 2004, Batista *et al.* 2007, Zafalon *et al.* 2007, Pinna *et al.* 2008, Scarparo *et al.* 2009). In the majority of these studies, the tissue response is assessed using traditional staining techniques (e.g. haematoxylin and eosin) and scoring systems. However, such methods rely on subjective assessment and have limited ability to identify the types of cells present. Although immunohistochemistry may provide valuable information by facilitating cellular identification and allowing evaluations of specific cellular responses, only a few studies have applied this technique to evaluate the biological properties of root canal sealers (Hemmi *et al.* 2003).

Taken together, it was hypothesized that different methacrylate resin-based root canal sealers elicit different inflammatory and immunological response patterns. In order to address the hypothesis, an assessment of the inflammatory/immunogenic potential of contemporary methacrylate resin-based root canal sealers was performed by means of subcutaneous

Table 1 Root canal sealers tested

Material	Batch no.	Manufacturer	Composition according to manufacturer
MetaSEAL	RS1	Parkell, Farmington, NY	Liquid: 4-META, HEMA and di-methacrylates Powder: zirconium oxide, silica and hydrophilic polymerization-initiator
Epiphany SE	149468	Pentron Clinical Technologies, Wallingford, CT	HEMA, Bis-GMA, calcium phosphate, 2,2-Bis (4-methacryloxypolyethoxyphenyl)propane, barium borosilicate glass, bismuth oxychloride, silica, allyl thiourea, benzoyl peroxide, photo-initiator, stabilizers and pigment
Super-Bond RC sealer	LX2	Sun Medical, Moriyama, Japan	Liquid: 4-META and methyl methacrylate Catalyst: tributylborane Powder: poly methyl methacrylate and zirconium oxide
Canals	5320R	Showa Yakuhin Kako, Tokyo, Japan	Powder: zinc oxide, barium sulfate, bismuth carbonate oxide and rosin Liquid: clove oil and olive oil

4-META, 4-methacryloxyethyl trimellitic anhydride; HEMA, 2-hydroxyethyl methacrylate; Bis-GMA, bisphenol A-glycidyl methacrylate.

© 2011 International Endodontic Journal

implantation followed by immunohistochemical detection of different inflammatory/immunocompetent cells.

Materials and methods

All experiments were approved and performed according to the guidelines of the Niigata University Intramural Animal Use and Care Committee.

The materials evaluated were MetaSEAL (Parkell, Farmington, NY, USA), Epiphany SE (Pentron Clinical Technologies, Wallingford, CT, USA), Super-Bond RC sealer (Sun Medical, Moriyama, Japan), and a zinc oxide-eugenol sealer (Canals; Showa Yakuhin Kako, Tokyo, Japan). Table 1 shows the composition of the test materials. Thirty 4-week-old male Wistar rats were used. Under anaesthesia with an intraperitoneal injection of 8% chloral hydrate (350 mg kg^{-1}), four 0.5-cm incisions were made on the back of each animal, 2 cm from the spine and at least 3 cm apart. Lateral undermining of the subcutaneous tissue produced four surgical cavities, which were equidistant from the centre of the animal's back.

Silicone tubes of approximately 3 mm in length, with an outer diameter of 3 mm and an inner diameter of 1 mm (Tigers Polymer Co, Osaka, Japan) were autoclaved. The sealers were mixed according to the manufacturers' recommendations and loaded into the silicone tubes. No light curing was performed. The tubes were then inserted into the surgical cavities; each animal received the four test materials, and the site of implantation was standardized. The incisions were then sutured with 4-0 silk thread (Mani, Tochigi, Japan). Solid silicone rods (Sanplatec Co, Osaka, Japan) of the same size were implanted in different animals and used as negative controls. Observations were performed at 7, 14 and 28 days after surgery (n = 8, each).

After the given periods, the animals were killed by anaesthetic overdose. The implants and their surrounding tissue were carefully removed in blocks and then fixed in 4% paraformaldehyde solution for 24 h. After fixation, the tissues were processed to produce frozen sections that were cut parallel to the long axis of the tubes. Serial sections of approximately 8 μm thick were obtained from the central part of each tube or silicone rod. They were then processed for haematoxylin–eosin (H-E) staining and immunohistochemistry. Immunoperoxidase staining was performed with the monoclonal antibodies OX6 (reactive to rat major histocompatibility complex (MHC) class II molecules (Fukumoto *et al.* 1982); Serotec, Oxford, UK; diluted 1 : 4000), W3/13 (mouse anti-rat CD43 reactive to neutrophils, plasma cells, and T-lymphocyte subsets (Barclay 1981); Serotec; diluted 1 : 200) and ED1 (mouse anti-rat CD68 reactive to macrophages (Dijkstra *et al.* 1985, van den Berg *et al.* 2001); Serotec; diluted 1:200) as primary antibodies. After blocking endogenous peroxidase activity with 0.3% hydrogen peroxide in methanol for 30 min, the sections were incubated with one of the primary antibodies at 4 °C overnight. After washing with phosphate-buffered saline, the sections were reacted with biotinylated horse anti-mouse IgG (Vector, Laboratories, Burlingame, CA, USA) for 2 h at room temperature and then with avidin–biotin–peroxidase complex (Elite ABC kit, Vector) for 30 min at room temperature. Immunoreactivity was visualized with the DAB Substrate Kit (Vector) and counterstained with methyl green. Negative control staining was performed by replacing the primary antibodies with phosphate-buffered saline.

The numbers of OX6, ED1 and W3/13-positive cells were enumerated in the areas in contact with each sealer and the corresponding areas of the controls. Representative sections were chosen from the most central cuts through the opening (one section per specimen), and the number of positively stained cells within a field (1.2 × 0.8 mm), which was placed just beneath the tube opening, was enumerated. Statistical differences were analysed with Friedman's test and Scheffe's test (comparisons between test materials), or Mann–Whitney's U-test test with Bonferroni's correction (test–control comparisons).

Results

Histologic findings

In each group, the implants were encapsulated by fibrous connective tissues, which were more evident with longer observation periods. The areas in contact with each sealer had varying degrees of inflammation characterized by the infiltration of macrophages, a relatively small number of neutrophils and occasional lymphocytes. Plasma cells and multinuclear giant cells were rarely seen. The inflammatory reaction subsided with time, but was still detectable at 28 days. In general, the controls showed less-intense inflammatory cell infiltration than the test materials.

Immunohistochemical findings

Negative control staining did not reveal any specific immunoreaction. OX6 and W3/13 caused membrane

© 2011 International Endodontic Journal

staining, whereas ED1 caused granular cytoplasmic staining, as reported previously (Cantrell *et al.* 1983, Dijkstra *et al.* 1985).

Morphology and distribution of cells positive to each antibody was in general similar in each material; thus, representative photomicrographs from a MetaSEAL specimen are shown in FIg. 1.

OX6-positive cells showing various morphologies (round, oval, slender and irregular) were distributed in the areas in contact with each sealer (Fig. 1b,f). These cells were predominantly identified as macrophages, but fibroblast-like cells, some endothelial cells and a small number of lymphocyte-like cells also demonstrated OX6-immunoreactivity. The accumulation of OX6-positive cells was frequently seen around the implanted materials, although cells with engulfed sealer particles in their cytoplasm did not react with OX6.

ED1 (CD68)-positive cells with round, oval or irregular morphologies were observed in the areas in contact with each sealer as well as around extruded materials (Fig. 1c,g). Cells that had phagocytosed sealer particles often showed strong ED1-immunoreactivity.

W3/13 (CD43)-positive cells were scattered in the area around the tube opening (Fig. 1d,h). The majority of these cells were identified as neutrophils, although mononuclear cells showing W3/13-immunoreactivity were occasionally encountered.

The control specimens did not differ from the test specimens with regard to the types of cells that were positive for each antibody, although the density of positively stained cells was generally lower in the controls than in the corresponding test specimens.

Cell count

Comparisons between the test materials demonstrated that canals showed a significantly higher number of W3/13-positive cells than MetaSEAL at 28 days ($P < 0.05$) (Fig. 2). There were no significant differences in the number of OX6- or ED1-positive cells between any of the test materials at any time period ($P > 0.05$).

Test–control comparisons of the number of OX6-positive cells revealed that no significant differences were detected at 7 days, but that significant differences were detected for two and three of the four sealers at 14 and 28 days, respectively. Regarding the number of ED1-positive cells, all the test materials at each time point, except for Epiphany SE at 28 days, showed significantly larger values than the corresponding controls. Significant test–control differences in the numbers of W3/13-positive cells were detected for all test materials at 7 days, but at 28 days significant test–control differences were only detected for Super-Bond RC sealer and Canals.

Discussion

In this study, the inflammatory and immunological response patterns of contemporary methacrylate resin-based root canal sealers was assessed by means of subcutaneous implantation followed by immunohistochemical detection of inflammatory/immunocompetent cells, under the hypothesis that different sealers elicit different patterns. In order to avoid the assessment criteria being too narrow, three monoclonal antibodies

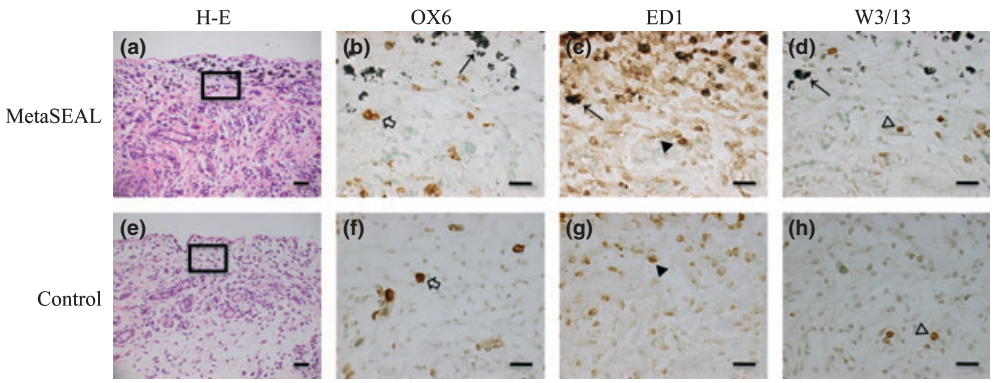

Figure 1 Tissue reaction to MetaSEAL (a–d) and the control (e–h) at 14 days. (a, e) H–E staining, bar = 100 μm. The boxes in a and e show the approximate locations of the fields in b–d and f–h, respectively. (b–d, f–h) Immunoperoxidase staining of OX6 (b, f), ED1 (c, g), and W3/13 (d, h); bar = 25 μm. Arrows, sealer particles; open arrows, OX6-positive cells; arrowheads, ED1-positive cells; and open arrowheads, W3/13-positive cells.

© 2011 International Endodontic Journal

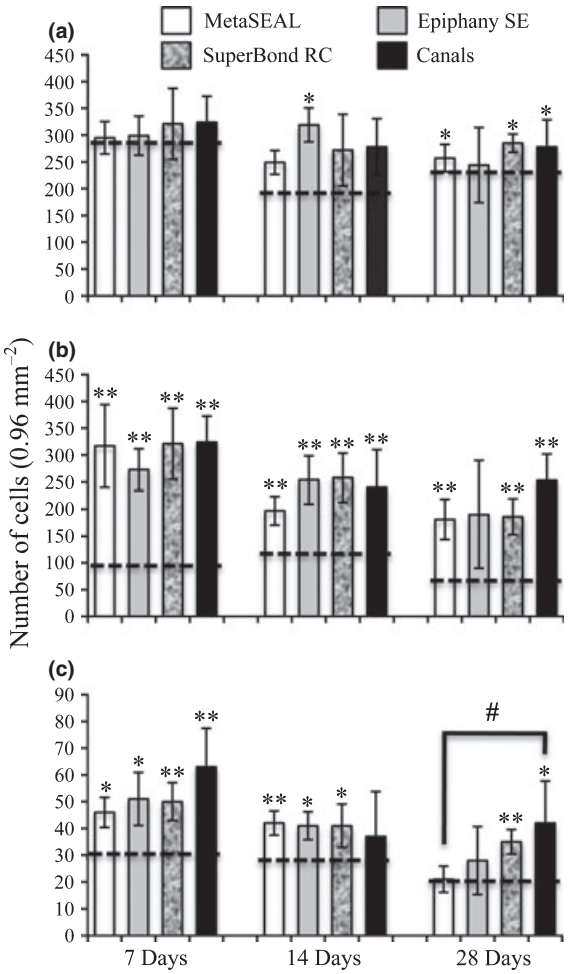

Figure 2 Mean and SD of the number of cells that are immunopositive for OX6 (a), ED1 (b), and W3/13 (c). Horizontal dotted lines indicate the mean value of the corresponding controls. No significant differences were detected amongst resin-based sealers at each time point for each type of cells. Test–control differences were most notable for ED1-positive cells #$P < 0.05$ (Friedman's test and Scheffe's test). *$P < 0.01$ and **$P < 0.001$ (vs. control; Mann–Whitney's *U*-test with Bonferroni's correction).

was selected, which allowed the detection of different types of inflammatory/immunocompetent cells. Using this method, the identification and counting of specific cell types may be much easier to achieve, and the results might be more objective, compared with assessment utilizing traditional subjective scoring systems and H–E-stained sections. Based on the findings, the hypothesis was rejected, as similar patterns were present as determined by the density of ED1-, OX6- and W3/13-positive cells.

To date, a limited number of studies have investigated the biological properties of self-adhesive methacrylate resin-based sealers. Studies have shown that MetaSEAL is less cytotoxic than nonetching methacrylate resin-based sealers (RealSeal and EndREZ) (Ames *et al.* 2009) and a zinc oxide–eugenol sealer (Pinna *et al.* 2008), but is more toxic than AH Plus, an epoxy resin-based sealer (Pinna *et al.* 2008). With regard to Epiphany SE, one study showed that it has similar cytotoxicity to original Epiphany (Gambarini *et al.* 2009). However, another study has demonstrated that RealSeal SE, a self-adhesive sealer that is compositionally similar to Epiphany SE, is less cytotoxic than RealSeal (Ames *et al.* 2009). Taken together, the cytocompatibility of self-adhesive methacrylate resin-based sealers may be comparable or better than those of previous generation nonetching resin sealers.

On the other hand, the Super-Bond RC sealer has been reported to show better cytocompatibility than several other types of sealers (Eldeniz *et al.* 2007). This is consistent with studies showing that the original Super-Bond resin cement possesses favourable cytocompatibility to dental pulp cells (Inoue *et al.* 2001, Fujisawa & Atsumi 2004) and an osteoblastic cell line (Yoshimine *et al.* 2007). Another study demonstrated that the subcutaneous tissue reaction to Super-Bond RC sealer was milder than that to a zinc oxide–eugenol sealer (Hemmi *et al.* 2003).

In the present study, inter-material comparisons revealed that MetaSEAL showed a significantly smaller number of W3/13-positive cells than Canals at 28 days. This may be attributable to the fact that zinc oxide–eugenol sealers cause prolonged neutrophil infiltration (Olsson & Wennberg 1985, Kolokuris *et al.* 1996, Scarparo *et al.* 2009).

In all of the sealers tested, the number of W3/13-positive cells (neutrophils) surrounding the sealer was relatively low without any marked accumulation at 7 days. However, all of the sealers induced the infiltration of significantly more W3/13-positive cells than the control at 7 days. Thus, it is apparent that they have the potential to cause neutrophil infiltration. Similar results have been reported in a number of studies (Görduysus *et al.* 1998, Bernáth & Szabó 2003, Zmener 2004, Sousa *et al.* 2006, Zafalon *et al.* 2007, Scarparo *et al.* 2009).

The test–control differences were most notable for ED1-positive cells; all the sealers at each time point, except for Epiphany SE at 28-days, showed significantly larger values than the corresponding controls. This suggests that these sealers primarily induce

© 2011 International Endodontic Journal

macrophage-dependent reaction(s) such as persistent foreign body reactions to extruded sealer particles.

OX6 antibody recognizes the MHC class II molecules expressed on several cell types such as macrophage subpopulations, dendritic cells, B cells, and activated T cells (Kaneko *et al.* 2001, Ozaki *et al.* 2004). MHC class II molecule–expressing cells act as antigen-presenting cells, which aid the initiation of T-cell responses by uptaking, processing and presenting antigens to T lymphocytes (Hart 1997). Thus, the level of MHC class II molecule expression may be considered as an indicator of a sealers' immunogenic potential. In this study, all the sealers exhibited infiltration by a significantly higher number of OX6-positive cells than the corresponding controls at 14 and 28 days. This finding suggests that these sealers continuously release antigenic substances that facilitate the recruitment of class II molecule–expressing cells and/or have the potential to directly or indirectly induce class II molecule expression.

At present, it is not clear which component(s) in each sealer possess the potential of causing the infiltration of inflammatory/immunocompetent cells. One potential candidate for the cyto- and/or tissue-irritation of methacrylate resin-based materials is unpolymerized hydrophilic monomers such as HEMA, as it has been reported that HEMA is cytotoxic (Bouillaguet *et al.* 1996), identified as an allergen of contact dermatitis (Rustemeyer & Frosch 1996) and possesses inflammatogenic and adjuvant properties (Sandberg *et al.* 2005). In the present study, however, there was no significant difference in the level of cellular infiltration amongst different resin-based materials, regardless of the presence or absence of HEMA. On the other hand, 4-META/MMA-TBB resin is known to induce macrophages accumulation when applied to vital exposed pulps, and soft-tissue hybrid layer created along the resin-pulp tissue interface has been suggested to provoke the macrophage infiltration (Nakamura *et al.* 2000). A similar mechanism would be involved in the subcutaneous tissue reaction to 4-META-containing methacrylate resin-based sealers.

Conclusions

All the methacrylate resin-based sealers tested showed a similar level of inflammatory/immunocompetent cell infiltration. MetaSEAL induced less intense neutrophil infiltration than Canals. Controls exhibited milder infiltration of inflammatory/immunocompetent cells compared with all the test materials.

References

Ames JM, Loushine RJ, Babb BR *et al.* (2009) Contemporary methacrylate resin-based root canal sealers exhibit different degrees of ex vivo cytotoxicity when cured in their self-cured mode. *Journal of Endodontics* **35**, 225–8.

Barclay AN (1981) The localization of populations of lymphocytes defined by monoclonal antibodies in rat lymphoid tissues. *Immunology* **42**, 593–600.

Batista RF, Hidalgo MM, Hernandes L, Consolaro A, Velloso TR, Cuman RK (2007) Microscopic analysis of subcutaneous reactions to endodontic sealer implants in rats. *Journal of Biomedical Materials Research. Part A* **81**, 171–7.

van den Berg TK, Döpp EA, Dijkstra CD (2001) Rat macrophages: membrane glycoproteins in differentiation and function. *Immunological Reviews* **184**, 45–57.

Bernáth M, Szabó J (2003) Tissue reaction initiated by different sealers. *International Endodontic Journal* **36**, 256–61.

Bouillaguet S, Wataha JC, Hanks CT, Ciucchi B, Holz J (1996) *In vitro* cytotoxicity and dentin permeability of HEMA. *Journal of Endodontics* **22**, 244–8.

Cantrell DA, Robins RA, Baldwin RW (1983) A comparison of membrane markers on rat cytotoxic cells. *Immunology* **49**, 139–46.

Dijkstra CD, Döpp EA, Joling P, Kraal G (1985) The heterogeneity of mononuclear phagocytes in lymphoid organs: distinct macrophage subpopulations in the rat recognized by monoclonal antibodies ED1, ED2 and ED3. *Immunology* **54**, 589–99.

Eldeniz AU, Mustafa K, Ørstavik D, Dahl JE (2007) Cytotoxicity of new resin-, calcium hydroxide- and silicone-based root canal sealers on fibroblasts derived from human gingiva and L929 cell lines. *International Endodontic Journal* **40**, 329–37.

Fujisawa S, Atsumi T (2004) Cytotoxicities of a 4-META/MMA-TBBO resin against human pulp fibroblasts. *Dental Materials Journal* **23**, 106–8.

Fukumoto T, McMaster WR, Williams AF (1982) Mouse monoclonal antibodies against rat major histocompatibility antigens. Two Ia antigens and expression of Ia and class I antigens in rat thymus. *European Journal of Immunology* **12**, 237–43.

Gambarini G, Romeo U, Tucci E *et al.* (2009) Cytotoxicity of epiphany SE endodontic sealer: a comparative *in vitro* study. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research* **15**, I15–8.

Geurtsen W, Leyhausen G (1997) Biological aspects of root canal filling materials – histocompatibility, cytotoxicity, and mutagenicity. *Clinical Oral Investigations* **1**, 5–11.

Görduysus MO, Etikan I, Gököz A (1998) Histopathological evaluation of the tissue reactions to Endo-Fill root canal sealant and filling material in rats. *Journal of Endodontics* **24**, 194–6.

© 2011 International Endodontic Journal

Hart DN (1997) Dendritic cells: unique leukocyte populations which control the primary immune response. *Blood* **90**, 3245–87.

Hemmi H, Baba T, Toda T (2003) Tissue compatibility of newly-developed resin root canal sealers. *Japanese Journal of Conservative Dentistry* **46**, 690–706.

Inoue T, Miyakoshi S, Shimono M (2001) The *in vitro* and *in vivo* influence of 4-META/MMA-TBB resin components on dental pulp tissues. *Advances in Dental Research* **15**, 101–4.

Kaneko T, Okiji T, Kan L, Takagi M, Suda H (2001) Ultrastructural analysis of MHC class II molecule-expressing cells in experimentally induced periapical lesions in the rat. *Journal of Endodontics* **27**, 337–42.

Kolokuris I, Beltes P, Economides N, Vlemmas I (1996) Experimental study of the biocompatibility of a new glass-ionomer root canal sealer (Ketac-Endo). *Journal of Endodontics* **22**, 395–8.

Nakamura M, Inoue T, Shimono M (2000) Immunohistochemical study of dental pulp applied with 4-META/MMA-TBB adhesive resin after pulpotomy. *Journal of Biomedical Materials Research* **51**, 241–8.

Olsson B, Wennberg A (1985) Early tissue reaction to endodontic filling materials. *Endodontics and Dental Traumatology* **1**, 138–41.

Ozaki A, Ishida W, Fukata K, Fukushima A, Ueno H (2004) Phenotypic changes and inflammatory cell distribution in the cornea during development of experimental immune-mediated blepharoconjunctivitis. *Japanese Journal of Ophthalmology* **48**, 333–9.

Pinna L, Brackett MG, Lockwood PE *et al.* (2008) *In vitro* cytotoxicity evaluation of a self-adhesive, methacrylate resin-based root canal sealer. *Journal of Endodontics* **34**, 1085–8.

Ricucci D, Langeland K (1998) Apical limit of root canal instrumentation and obturation: part 2 A histological study. *International Endodontic Journal* **31**, 394–409.

Rustemeyer T, Frosch PJ (1996) Occupational skin diseases in dental laboratory technicians. (I). Clinical picture and causative factors. *Contact Dermatitis* **34**, 125–33.

Sandberg E, Kahu H, Dahlgren UI (2005) Inflammatogenic and adjuvant properties of HEMA in mice. *European Journal of Oral Sciences* **113**, 410–6.

Sari S, Duruturk L (2007) Radiographic evaluation of periapical healing of permanent teeth with periapical lesions after extrusion of AH Plus sealer. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **104**, e54–9.

Scarparo RK, Grecca FS, Fachin EVF (2009) Analysis of tissue reactions to methacrylate resin-based, epoxy resin-based, and zinc oxide-eugenol endodontic sealers. *Journal of Endodontics* **35**, 229–32.

Schwartz RS (2006) Adhesive dentistry and endodontics. Part 2: bonding in the root canal system-the promise and the problems: a review. *Journal of Endodontics* **32**, 1125–34.

Seltzer S (1999) Long-term radiographic and histological observations of endodontically treated teeth. *Journal of Endodontics* **25**, 818–22.

Siqueira JF Jr (2001) Strategies to treat infected root canals. *Journal of the California Dental Association* **29**, 825–37.

Sousa CJA, Montes CRM, Pascon EA, Loyola AM, Versiani MA (2006) Comparison of the intraosseous biocompatibility of AH Plus, EndoREZ, and Epiphany root canal sealers. *Journal of Endodontics* **32**, 656–62.

Sundqvist G, Figdor D (1998) Endodontic treatment of apical periodontitis. In: Ørstavik D, Pitt Ford TR, eds. *Essential Endodontology. Prevention and Treatment of Apical Periodontitis*. Oxford, UK: Blackwell Science, pp. 242–77.

Yoshimine Y, Ono M, Akamine A (2007) *In vitro* comparison of the biocompatibility of mineral trioxide aggregate, 4META/MMA-TBB resin, and intermediate restorative material as root-end-filling materials. *Journal of Endodontics* **33**, 1066–9.

Zafalon EJ, Versiani MA, de Souza CJ, Moura GC, Dechichi P (2007) *In vivo* comparison of the biocompatibility of two root canal sealers implanted into subcutaneous connective tissue of rats. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics* **103**, e88–94.

Zmener O (2004) Tissue response to a new methacrylate-based root canal sealer: preliminary observations in the subcutaneous connective tissue of rats. *Journal of Endodontics* **30**, 348–51.

© 2011 International Endodontic Journal

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy.
Users should refer to the original published version of the material.