doi:10.1111/j.1365-2591.2007.01329.x

Histological, ultrastructural and quantitative investigations on the response of healthy human pulps to experimental capping with mineral trioxide aggregate: a randomized controlled trial

P. N. R. Nair[1], H. F. Duncan[2], T. R. Pitt Ford[2] & H. U. Luder[1]

[1]Institute of Oral Biology, Section of Oral Structures and Development, Centre of Dental and Oral Medicine, University of Zurich, Zurich, Switzerland; [2]Dental Institute, Department of Conservative Dentistry, King's College London, London, UK

Abstract

Nair PNR, Duncan HF, Pitt Ford TR, Luder HU. Histological, ultrastructural and quantitative investigations on the response of healthy human pulps to experimental capping with mineral trioxide aggregate: a randomized controlled trial. *International Endodontic Journal*, **41**, 128–150, 2008.

Aim To investigate the pulpal response to direct pulp capping in healthy human teeth with mineral trioxide aggregate (MTA) as against calcium hydroxide cement (Dycal®) as control.

Methodology Twenty healthy human third molars had iatrogenic pulpotomy and direct pulp capping with MTA. Another 13 teeth were capped with Dycal® as controls. The teeth were restored, with IRM®, clinically reviewed and extracted after a number of pre-determined intervals (1 week, 1 month and 3 months). The specimens were fixed, decalcified, subdivided axially into two halves in the oro-buccal (lingual–buccal) plane, embedded in plastic, serial sectioned and evaluated qualitatively and quantitatively by correlative light and transmission electron microscopy with appropriate statistical evaluation of the quantitative data.

Results Iatrogenic pulpal wounds treated with MTA were mostly free from inflammation after 1 week and became covered with a compact, hard tissue barrier of steadily increasing length and thickness within 3 months following capping. Control teeth treated with Dycal® revealed distinctly less consistent formation of a hard tissue barrier that had numerous tunnel defects. The presence of pulpal inflammation up to the longest observation period (3 months) after capping, was a common feature in Dycal® specimens.

Conclusions The MTA was clinically easier to use as a direct pulp–capping agent and resulted in less pulpal inflammation and more predictable hard tissue barrier formation than Dycal®. Therefore, MTA or equivalent products should be the material of choice for direct pulp capping procedures instead of hard setting calcium hydroxide cements.

Keywords: calcium hydroxide, dentine bridge, MTA, pulp capping.

Received 29 March 2007; accepted 25 July 2007

Introduction

Preserving the dental pulp or part of it in a healthy state is important in treating teeth with exposed vital pulp, particularly in those where the root formation has not yet been completed. Exposure of dental pulps can happen as a result of caries, trauma or cavity preparation. Direct pulp capping has been one of the treatment options with a long tradition. It is a procedure in which the exposed pulp is covered with a protective wound dressing or base placed directly over the site of exposure (ESE 2006). Ideally, the pulp heals with the formation of a mineralized tissue barrier across the exposed surface, commonly referred to as dentine bridge. Historically, a variety of materials and medicaments have been used often with disappointing

Correspondence: Dr P. N. R. Nair, BVSc, DVM, PhD (Hon), Senior Scientist, Institute of Oral Biology, Section of Oral Structures and Development (OSD), Centre of Dental & Oral Medicine, University of Zurich, Plattenstrasse 11, CH-8032 Zurich, Switzerland (Tel.: 0041 44 634 31 42; fax: 0041 44 312 32 81; e-mail: ram.nair@zzmk.uzh.ch).

results such that the exposed pulp came to be considered a doomed organ (Rebel 1922) and the concept of dentine bridge as a myth (Langeland *et al.* 1973).

The introduction of calcium hydroxide [$Ca(OH)_2$] into dentistry (Hermann 1930) and its popularization as a pulpal wound-dressing agent (Hermann 1936) resulted in renewed interest in pulp-capping. The effect of $Ca(OH)_2$ on exposed pulp has been extensively researched (Glass & Zahnder 1949, Nyborg 1955, Schröder 1972, 1973a, 1978, Stanley & Lundy 1972, Tronstad 1974, Cvek *et al.* 1987, Pitt Ford & Roberts 1991) with the result that $Ca(OH)_2$ has become the agent of choice for pulp capping. However, the outcome of pulp capping with $Ca(OH)_2$ remains unpredictable, possibly because of the importance of direct contact between the sealant and the pulp tissue without any intervening blood clot (Schröder 1973a,b). The latter is generally a difficult pre-requisite to achieve under clinical conditions. During the past three decades a number of publications appeared on pulp capping in humans using $Ca(OH)_2$, bonding agents and other materials, including mineral trioxide aggregate (MTA). The papers have been systematically reviewed (Olsson *et al.* 2006) to evaluate available evidence on the formation of hard tissue barrier after pulp capping. In total 107 studies were identified of which only 21 met the selection criteria for evaluation. None of the 21 publications was assessed to have a high level of evidence, one was of moderate quality and the remaining 20 provided only low level of evidence.

Mineral trioxide aggregate is a relatively novel pulp sealing agent that has been originally reported to close communication between the pulp canal system and external surfaces of the teeth (Lee *et al.* 1993). MTA is essentially Portland cement with, for radiopacity, bismuth oxide in 4 : 1 proportions (Camilleri *et al.* 2005). Commercial MTA is available (ProRoot® MTA; Tulsa Dental Products, Tulsa, OK, USA) in grey and white forms. The composition of the two products differs slightly in that the white MTA, free from iron, is composed mainly of tricalcium silicate and bismuth oxide whilst grey MTA also contains dicalcium silicate.

The MTA has been well studied in experiments that showed good sealing ability (Torabinejad *et al.* 1993) and bio-compatibility (Holland *et al.* 1999a). It has been successfully used for retrograde filling of root ends (Torabinejad *et al.* 1995) and to close iatrogenic perforations of roots (Lee *et al.* 1993) and furcations (Pitt Ford *et al.* 1995). Attempts have also been made to use it as a root-canal filling material in dogs (Holland *et al.* 1999b). Dentine bridge-like hard tissue was consistently observed when MTA was used as a pulp–capping agent in monkeys (Pitt Ford *et al.* 1996), dogs (Tziafas *et al.* 2002, Asgary *et al.* 2006) and rats (Salako *et al.* 2003). It is essential to test the pulp healing ability of MTA on human teeth before its routine clinical application (ISO 1997). Except for few studies on the clinical and radiographical outcomes of direct pulp capping with MTA on human primary (Farsi *et al.* 2005, Maroto *et al.* 2005, 2006) and permanent (Farsi *et al.* 2006) teeth, there seem to be only four publications in the literature (Aeinehchi *et al.* 2003, Caicedo *et al.* 2006, Chacko & Kurikose 2006, Iwamoto *et al.* 2006), in which the response of human pulp to MTA has been histologically studied. The results of the latter studies on humans corroborated those reported in animals.

Table 1 Summary of the clinical data of subjects and teeth involved

No.	Code	Patient	Age	Sex	Tooth	Group	Observation
01	MTA-01	NB	25	M	28	MTA	1 week
02	MTA-03	JM	28	M	38	MTA	1 week
03	MTA-05	LO	25	F	28	MTA	1 week
04[a]	MTA-06	DH	18	M	48	MTA	1 week
05	MTA-08	CC	24	F	18	MTA	1 week
06	MTA-09	MW	26	M	18	MTA	1 week
07	MTA-11	NS	24	M	28	MTA	1 week
08	MTA-22	ES	20	M	28	MTA	1 month
09	MTA-24	MM	22	M	28	MTA	1 month
10	MTA-25	GT	29	F	28	MTA	1 month
11	MTA-26	TJ	28	M	28	MTA	1 month
12	MTA-28	KP	20	F	48	MTA	1 month
13	MTA-29	LC	22	F	28	MTA	1 month
14	MTA-41	MT	19	F	28	MTA	3 months
15	MTA-43	LV	21	F	38	MTA	3 months
16[a]	MTA-44	EO	30	F	18	MTA	3 months
17[a]	MTA-45	VA	29	F	38	MTA	3 months
18	MTA-48	FB	19	F	18	MTA	3 months
19	MTA-50	KK	27	M	18	MTA	3 months
20	MTA-51	DH	23	F	28	MTA	3 months
21	MTA-02	NB	25	M	18	Dycal	1 week
22	MTA-04	JM	28	M	28	Dycal	1 week
23	MTA-07	CC	24	F	48	Dycal	1 week
23	MTA-10	NS	24	M	18	Dycal	1 week
25	MTA-23	ES	20	M	18	Dycal	1 month
26	MTA-27	KP	20	F	18	Dycal	1 month
27	MTA-30	LC	22	F	18	Dycal	1 month
28	MTA-31	DB	23	F	18	Dycal	1 month
29	MTA-32	AS	28	F	28	Dycal	1 month
30	MTA-42	KK	30	M	28	Dycal	3 months
31	MTA-46	VA	29	F	28	Dycal	3 months
32	MTA-47	AS	28	F	18	Dycal	3 months
33	MTA-49	FB	19	F	28	Dycal	3 months

[a]These specimens could not be processed fully due to technical reasons and did not yield results.

The data from the literature indicate MTA as a potential pulp–capping agent. However, the composition of the cell population seeding at the MTA–pulp interface, the fine structure of the interface and measurements of the hard tissue barrier formed at various stages after MTA-capping are unknown. These basic data are essential not only to judge the quality of pulpal healing and sealing but also helpful to design further studies on MTA-induced intercellular signalling and healing mechanisms of wounded pulps at a molecular level. The purpose of this study was to investigate, using correlative light and transmission electron microscopy (TEM), the short- and medium-term pulpal response to iatrogenic pulp capping with MTA (White Proroot® MTA; Dentsply, Tulsa Dental, Konstanz, Germany) in permanent human teeth.

Material and methods

Approval to conduct the study was granted from the United Kingdom National Health Service, Local Research Ethics Committee of Guy's and St Thomas' Hospitals (International Standard Randomised Controlled Trial Number 34146674).

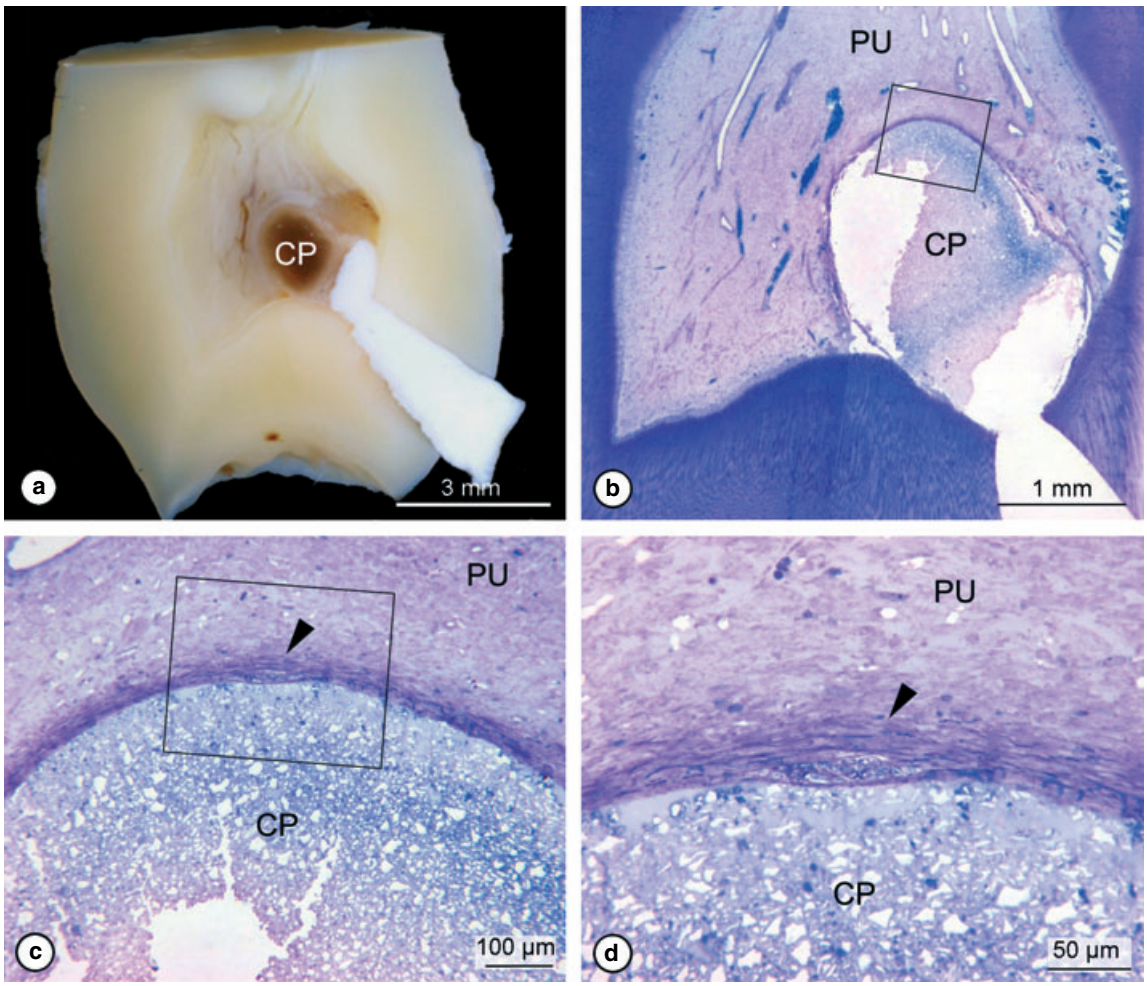


Figure 1 Pulpal response to mineral trioxide aggregate (MTA) capping after 1 week observation. Distal macrophotographic view (a) of the cut face of the mesial half of a maxillary left third molar (tooth 28) with remnants of the capping (CP) and restorative material (white plug in the cavity preparation and pulp chamber). The photomicrograph (b) represents part of a histological section of the specimen in (a). Note the cavity opening into the pulp chamber, remnants of the capping material (CP) and healthy remaining pulp (PU). The rectangular areas demarcated in (b) and (c) are magnified in (c) and (d) respectively. Note the pulp–cap interface with fibrous encapsulation (arrowheads) and absence of pulpal inflammation. Original magnifications: a ×7, b ×18, c ×90, d ×220.

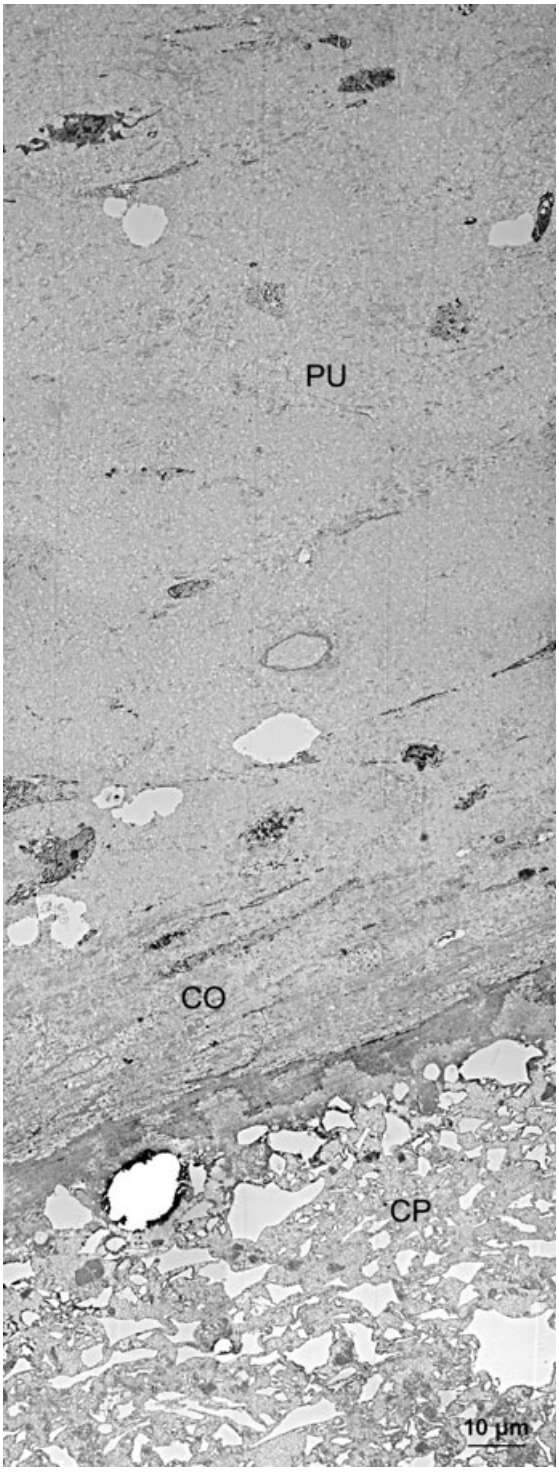


Figure 2 Correlative composite transmission electron micrograph of the pulp–cap junction shown in Fig. 1. The pulp (PU) subjacent to the capping material (CP) is totally devoid of inflammatory cells but rich in extracelluar matrix with scattered fibroblast-like pulpal cells. The pulp–cap interface shows no lining cells but fibrous encapsulation (CO) with fibre bundles running parallel to the interface. Original magnification: ×800.

Subjects and specimens

Young adult male and female patients (aged 18–30 years) considered for this randomized controlled study had clinically healthy third molars (erupted mandibular/maxillary) scheduled for extraction. All the subjects were treated in accordance with the Helsinki-declaration. Briefly, informed consent of each patient was obtained in writing after thoroughly explaining the clinical procedures, risks involved and clarifying all questions raised by the patients. The patient participation was voluntary and they were not financially remunerated for participation in the study. All the patients (male or female), were recruited by one operator. Teeth were excluded that had detectable caries, a previous restorative history or were not able to be isolated using rubber dam.

Clinical procedures

Tooth sensitivity was assessed using thermal and electric stimuli (Vitality Scanner Model 2006; Analytic Technology, Redmond, WA, USA). The teeth were isolated with cotton wool and dried prior to a −50 °C thermal (Endofrost®; Roeko, Langenau, Germany) and electric pulp test (EPT). The EPT reading was compared with that of the corresponding tooth on the same arch or if this was not possible with the tooth immediately mesial to this. Local anaesthetic (2% lidocaine hydrochloride with epinephrine 1 : 80 000; Septodont, Maidstone, UK) was administered around the tooth/teeth that were being treated in the experiment. The teeth were isolated with rubber dam and the area (tooth, dam and clamp) swabbed with 3% hydrogen peroxide followed by 0.2% chlorhexidine prior to a class V cavity being prepared. The maxillary third molars cavities were accessed from a palatal aspect and mandibular molars were accessed from a buccal aspect. The cavity size was standardized 3 × 2 mm (width by height). The depth of the cavity varied from tooth to tooth depending on the size of the pulp chamber but was generally around 4–5 mm. Drilling was performed intermittently using a tungsten carbide bur (Jet FG2058; Kerr, Salerno, Italy) in a high speed handpiece under waterspray until the cavity was in the vicinity of the pulp.

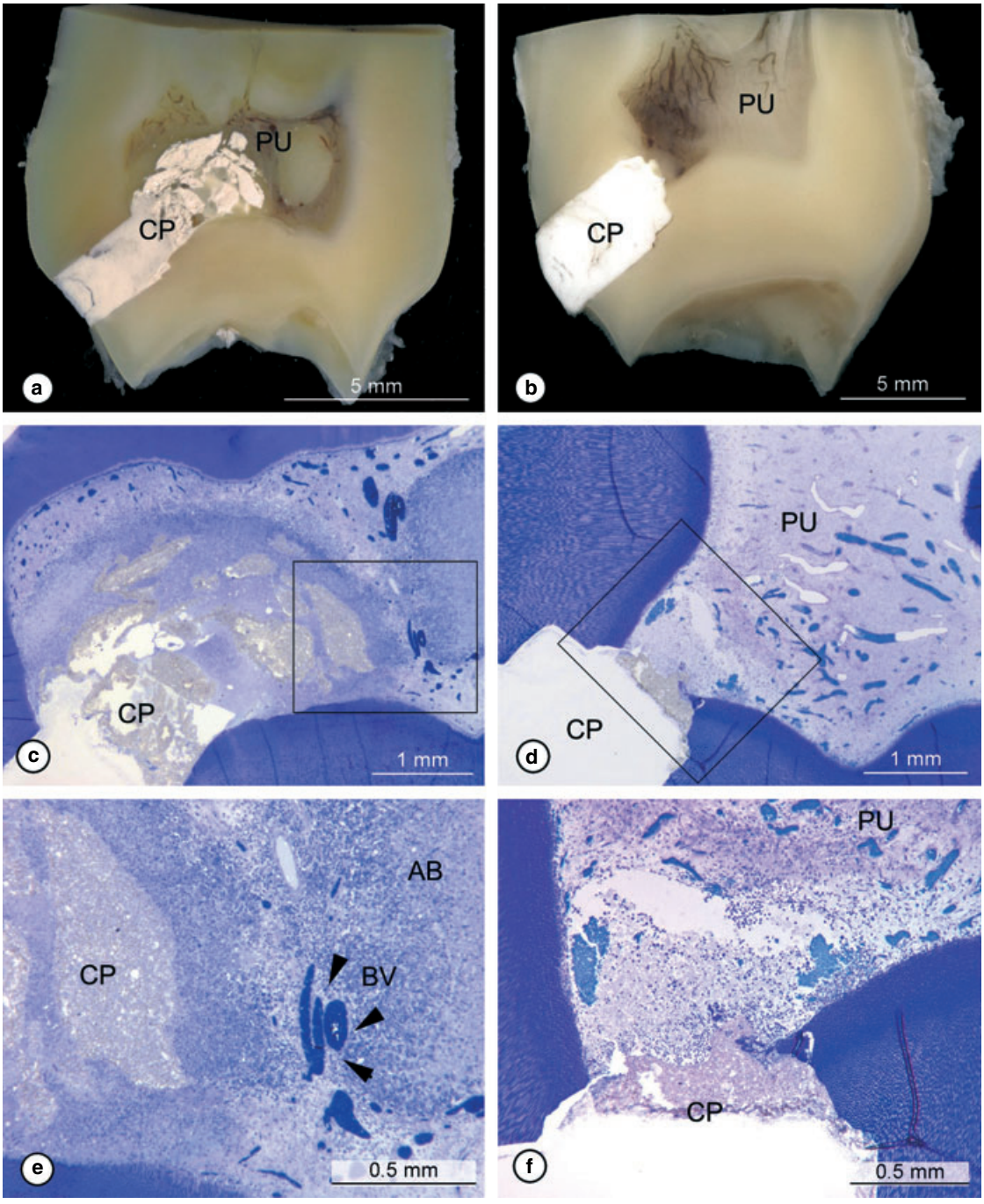


Figure 3 Examples of severe (a, c, e) and moderate (b, d, f) inflammatory pulpal response to Dycal® capping after 1 week observation. Mesial macrophotographic view (a) of the distal half of a maxillary left third molar (tooth 28) reveals remnants of the restorative and capping material (CP) as a white plug in the cavity preparation and pulp chamber. The photomicrograph (c) represents part of a histological section of the specimen in (a). The area demarcated in (c) is magnified in (e). Note the cavity opening into the pulp chamber, remnants of the capping material (CP) and acute pulpal response with abscess (AB) formation and dilatation of blood vessels (BV). The right column (b, d, f) shows the distal view of the mesial half of a maxillary right third molar (tooth 18) in (b) and corresponding photomicrographs in (d) and (f). Note the absence of any hard tissue barrier at the cap–pulp interface and moderate pulpal (PU) response to the capping material (CP in d, f). Original magnifications: a ×6, b ×4, c ×15, d ×17, e ×45, f ×40.

Deepest penetration was carried out with a long-shank round bur (191140 Meissinger; Neuss, Germany) in a slow speed handpiece. Pulpal exposure was made with a sharp explorer and standardized as closely as possible to a size between 0.5 and 1 mm. Salivary contamination was prevented by use of rubber dam and a sealing agent (Oroseal®; Ultradent, South Jordan, UT, USA). The cavity was irrigated with a 1% solution of sodium hypochlorite (Milton solution®; Milton Pharmaceutical UK, Bournemouth, UK) and haemostasis obtained by laying paper points soaked in sterile saline across the exposure. The pulp capping medicament used was either experimental White MTA (Proroot®; Dentsply, Tulsa Dental – batch number 02093081) or Dycal® Ivory (Dentsply Caulk, Milford, DE, USA – batch number 030805).

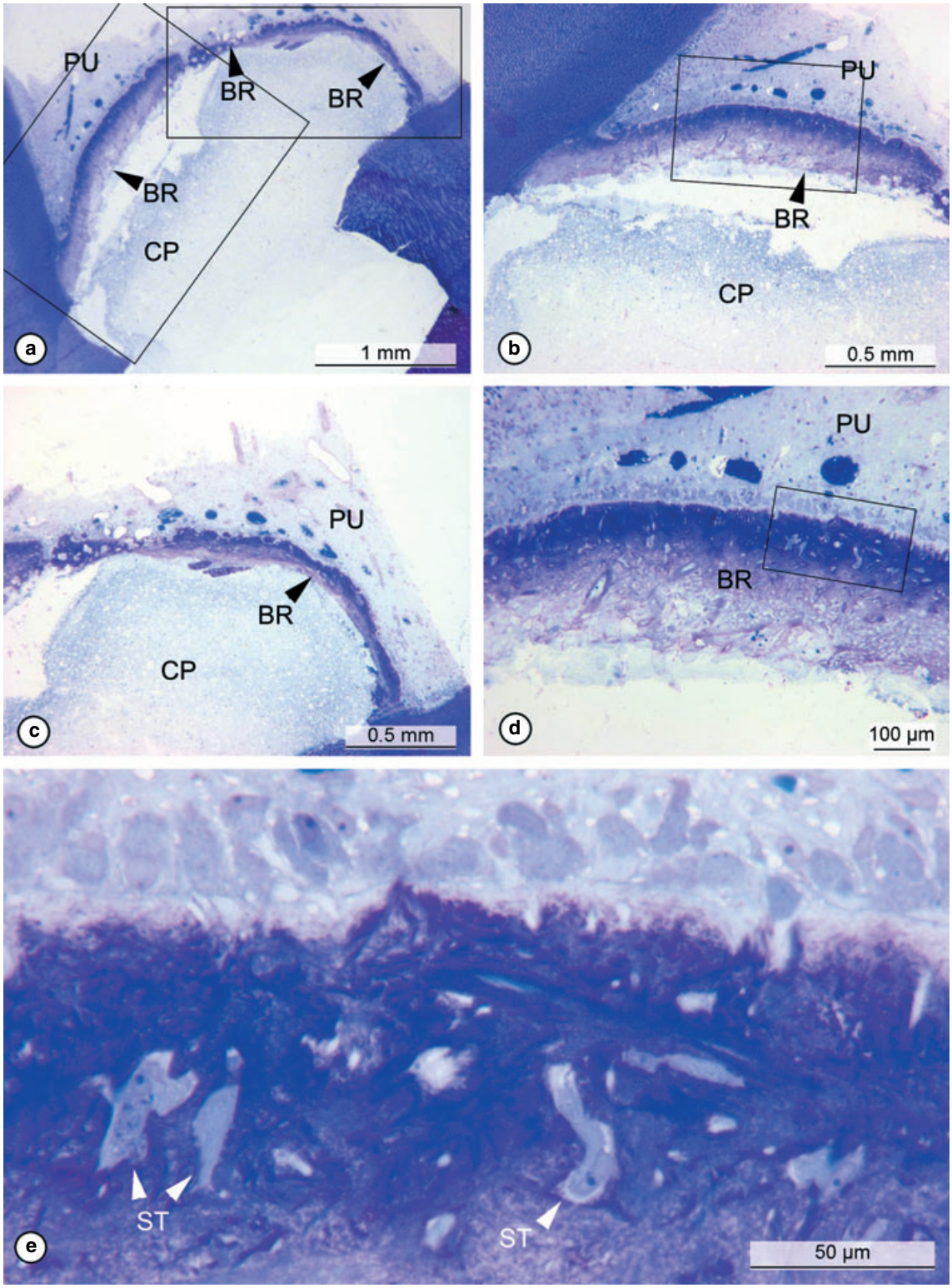
A total of 35 teeth from 23 patients were included in the study, of which 33 were histo-morphologically processed. The clinical and treatment data on the 33 teeth are presented in Table 1. One operator carried out all clinical procedures and material choice was decided randomly. This was achieved by a second person writing either experimental or control on a piece of paper and placing this into an envelope before sealing it. The appropriate number of experimental and control samples for each time interval had been decided beforehand. When two teeth were used in one patient, one was experimental and the other was control. The Dycal® paste was mixed in 1 : 1 ratio (v/v) according to the manufacturer's instructions and placed over the exposure site with an applicator. Using a stiff metal spatula MTA was mixed with the water supplied by the manufacturer in 3 : 1 ratio (v/v) and applied over the exposure site using an MTA applicator. Cavities were restored with Intermediate Restorative Material® (IRM; Dentsply DeTrey, Konstanz, Germany) which was mixed according to the manufacturer's instructions. The IRM was placed with a flat plastic instrument in a thickness of at least 4 mm and filled the remainder of the cavity.

The extractions were timed to occur after a number of pre-determined short- and medium-term intervals (1 week, 1 month and 3 months). All control and experimental teeth were carefully followed up and clinically assessed before extraction. This assessment included a history of symptoms (if any) and an examination recording tenderness to percussion and response to sensitivity test. These thermal and EPT tests were carried out in exactly the same manner as preoperatively. The teeth were extracted as atraumatically as possible, without sectioning of the teeth and damage to the pulp–capping interface by two designated oral surgeons. Where appropriate a muco-periosteal flap was reflected and buccal/distal bone was removed to facilitate their extraction. Immediately after extraction, the teeth were briefly rinsed in cold water to remove any blood which might interfere with fixative penetration. The roots were cut off at mid-root level – to allow fixative penetration – using a diamond coated bur and placed in individual screw-cap bottles containing 15 mL of half strength Karnovsky's solution (Karnovsky 1965), consisting of 2% paraformaldehyde and 2.5% glutaraldehyde buffered in 0.02 mol L^{-1} sodium cacodylate. The specimens were coded for blind histo-morphological processing and examination. They were stored in the fixative at 4 °C for a period up to 3 weeks prior to processing.

Tissue processing for light microscopy

The specimens were removed from the fixative solution, rinsed in water and demineralized in an ethylenediamine-tetraacetic acid solution (0.25 mol L^{-1} solution with 4% glutaraldehyde). Thereafter, they were subdivided into two halves in the axial plane using a sharp razor blade held by hand and with the aid of a stereomicroscope Wild M 8 (Wild, Heerbrugg, Switzerland). Special effort was taken to place an incision in such a way that the section-plane passed through the pulp cap in the oro-buccal (lingual–buccal) plane. The two halves of the specimen were macro photographed with a Leica M 420 Stereomicroscope (Leica Microsystems AG, Glattbrugg, Switzerland) equipped with the Nikon camera DS-5 M (Nikon Corporation, Tokyo, Japan).

The specimens were washed overnight in 0.185 mol L^{-1} sodium cacodylate buffer (pH 7.4), post-fixed for 3 h in 1.33% osmium tetraoxide buffered in 0.067 mol L^{-1} s-collidine (2,4,6 trimethylpyridine) (Bennett & Luft 1959), dehydrated in ascending grades of ethanol and embedded in Epon® (Fluka AG, Buchs, Switzerland). From each Epon® block, 1–2 μm thick survey sections and from selected blocks serial sections were prepared using glass or histodiamond knives (Diatome AG, Biel, Switzerland) and the ultramicrotome Reichert OM-U2 (Leica Microsystems, Glattbrugg, Switzerland). The sections were stained in periodic acid-schiff and methylene blue-Azur II and photomicrographed in a Dialux 20 photomicroscope (Leica Microsystems) equipped with the digital camera Progress C14 (Jenoptik, Eching, Germany) and a digital



imaging system (ImageAccess, Imagic, Glattbrugg, Switzerland). The sections were studied thoroughly in a light microscope for the following features: (i) hard tissue barrier formation: complete/incomplete; thickness; quality and presence of tunnel defects (Cox *et al.* 1996); (ii) interface between the barrier and soft tissue; (iii) nature of any inflammation and infiltrating cells in the pulp tissue; (iv) presence of microbial infection or histological signs of possible infection.

Counting of cells

Three random, more or less equidistant, sections were selected from each tooth specimen for quantitative analysis of extravascular residential and infiltrating cells at the capped pulpal front. In teeth with no bridge formation the site of the analysis consisted of the area immediately subjacent to the cap–pulp interface. In such specimens, the interface was either covered (encapsulated) with collagen fibre-bundles that ran parallel to the pulpal surface or exposed to the capping material in an ulcerative manner (open). The site of cellular analysis in the specimens with bridge formation included the tissue immediately subjacent to the bridge. As the pulpal cells that lined the bridge were assumed to be involved in the formation of the bridge, they were considered to be part of the bridge as far as cellular analysis was considered. The rest of the pulpal cell population that was counted consisted of fibroblasts, neutrophilic granulocytes, round cells (lymphocytes and plasma cells), macrophages and giant cells. Rarely encountered neural elements and other cells were categorized as 'others'. Each section was mounted onto a Wild M501 sampling microscope (Wild, Heerbrugg, Switzerland; Weibel 1970) equipped with a stereological test system of 110 × 110 mm on a screen. Cells were counted in three microscopic fields of 0.04 mm^2, each projected onto the screen using an oil immersion objective (50×) that gave a final magnification of approximately ×510. In addition to the cell counts, it was recorded, whether a particular microscopic field was located subjacent to an open, encapsulated, or bridged pulp–cap interface. Numbers determined primarily per sampling field were subsequently transformed into proportions of all cells present.

Measuring the bridges

Digital light micrographs with a resolution of 1300 × 1030 pixels depicting the entire pulp defect in the three sections selected for cell counting were taken at magnifications from ×5 to ×13 as described above. Blind regarding the antecedent capping procedure, these images were analysed with the program SIGMASCAN PRO 5.0 (SPSS, Chicago, IL, USA). Following calibration with respect to the microscopic magnification, the profile and approximate midaxis of an eventual bridge as well as any open cap–pulp interface were traced. Small patches of relatively homogeneous appearance, that sometimes occurred in the vicinity of encapsulated interfaces, were considered early bridges, although it could not be ascertained, whether they had been mineralized. Tracings served to measure the area of the bridge profile as well as the lengths of the bridge midaxis and the open interface. In case of a discontinuous bridge, data were assessed separately for each constituent part and summed up. From the primary measurements, the mean bridge thickness was calculated by dividing the profile area by the midaxis length, the total defect length as the sum of the midaxis and open interface lengths, and the relative bridge length by dividing the midaxis length by the total defect length.

Correlative transmission electron microscopy

A site of interest was identified from the pulp–cap interface by thoroughly studying the survey sections of the tooth directly in a light microscope and on the photomicrographs that were taken. Epon® blocks were first modified using the machine Leica EMTrim (Leica Microsystems) by preparing miniature pyramids of about 0.1–0.2 mm height at the site. The trimmed specimens were thin sectioned to a thickness of 60–80 nm using diamond knives (Diatome) in the ultramicrotome Reichert OM-U2 (Leica Microsystems). The

Figure 4 Pulpal response to mineral trioxide aggregate (MTA) capping after 1 month observation. Photomicrograph (a) is a bucco-lingual low magnification view of a maxillary left third molar (tooth 28) with remnants of the restorative and capping material (CP) in the cavity preparation and pulp chamber. Note the mineralized hard tissue barrier or bridge (BR) stretching across the full length of the exposed pulp. The rectangular areas demarcated on the left and right in (a) are magnified in (b) and (c) respectively. The micrographs (d) and (e) are magnifications of the areas demarcated in (b) and (d) respectively. Note the cuboidal pulpal cells lining the hard tissue barrier (in d, e) and absence of pulpal inflammation. Islands of pulpal soft tissue (ST in e) are enclosed within the hard tissue barrier. Original magnifications: a ×24, b, c ×36, d ×85, e ×600.

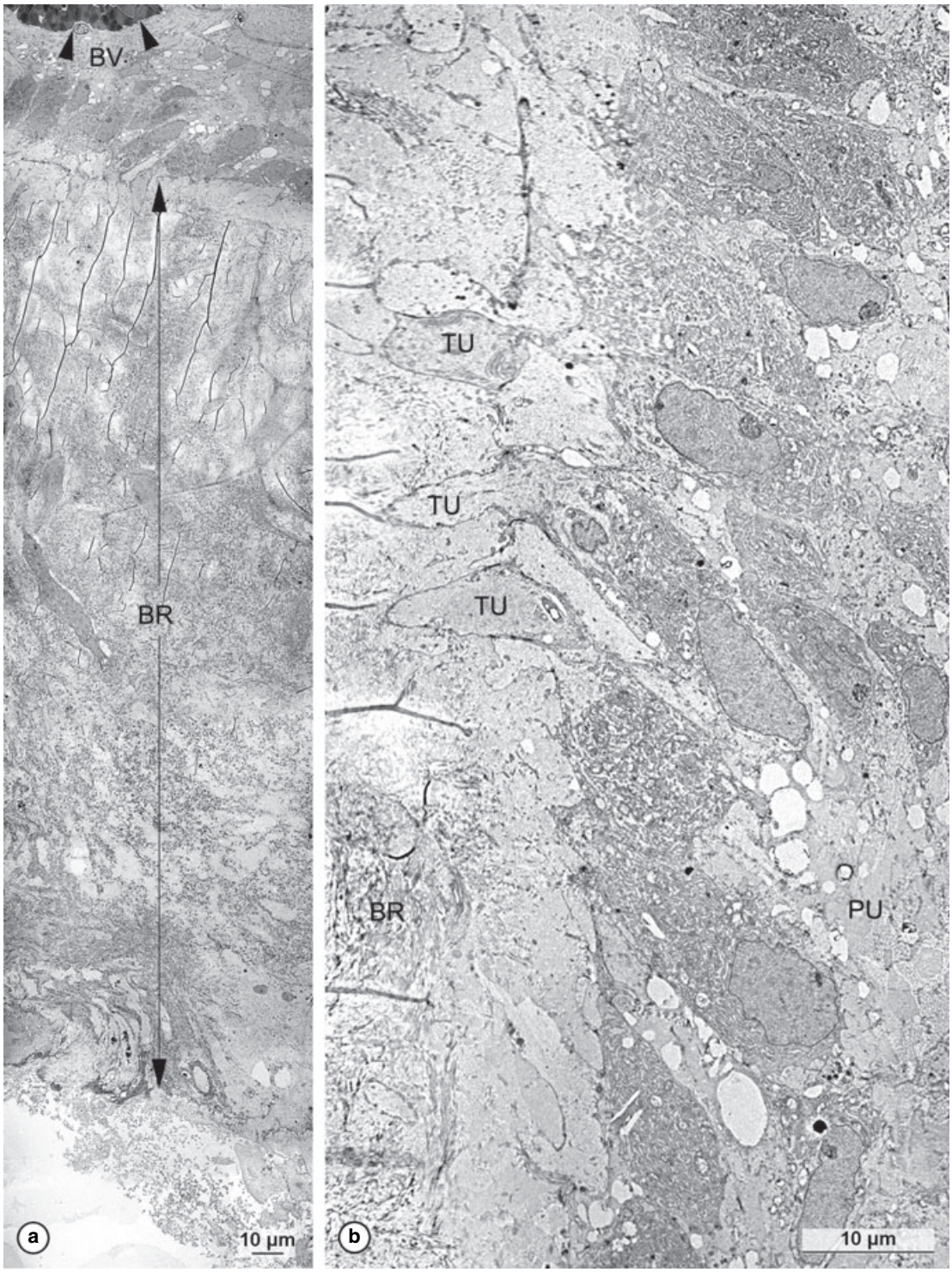


Figure 5 Correlative composite low magnification (a) transmission electron micrograph of the hard tissue bridge (BR) and subjacent pulp–cap junction shown in Fig. 4. The bridge–pulp interface in (a) is magnified and turned 90°clockwise in (b). The thick hard tissue barrier is lined on the pulpal side (PU) by columnar cells. Note the tubule-like (TU) structures into which cytoplasmic processes of the pulpal lining cells project. BV = blood vessel. Original magnification: a ×500, b ×22 000.

thin sections were collected on copper grids and double contrasted using lead and uranium salts (Fraska & Parks 1965, Venable & Coggeshall 1965). Thereafter, the specimens were washed in distilled water, dried and examined using the TEM EM 400T (Philips, Eindhoven, The Netherlands) at an accelerating voltage of 60 kV. Particular attention was paid to the fine detail of the cells that lined the newly formed bridge, any differentiation of the cell morphology with time intervals, any evidence of tubular structure and the content of these tubules, and areas which harboured microorganisms. Digital electron micrographs were captured at a resolution of 2272×2272 pixels using a Hamamatsu ORCA-HR camera (Hamamatsu Photonics, Hamamatsu City, Japan) and the program AMT IMAGE CAPTURE ENGINE 5.42 (Advanced Microscopy Technics, Danvers, MA, USA).

Statistical analysis

For representation of the quantitative data, results obtained from the three sections belonging to one specimen were pooled. In case of the cell counts, data derived from sampling fields subjacent to a hard tissue bridge and open or encapsulated cap–pulp interface were kept separate. Statistical tests were made using factorial analysis of variance with repeated measurements. Individual sections and, in the case of cell counts, sampling fields were treated as repeated measurements, whilst the treatment procedures [$Ca(OH)_2$ vs. MTA] and observation periods (1 week, 1 month and 3 months) were included as factors. The program SPSS 9.0 (SPSS) served for calculating statistical means and ranges as well as for performing the analyses of variance.

Results

A total of 35 teeth were included in the study, of which 33 were histologically processed (Table 1). Two teeth were excluded, because one of them had dentine caries that was not identified until after the tooth was extracted, and the other fractured during the extraction procedure. Of the 33 remaining teeth, three did not yield any morphological information because of technical reasons. Clinical status of the 33 teeth during the period from pulp-capping to extraction, determined by a combination of physical examination and tooth sensitivity tests, revealed that all the teeth were asymptomatic and sensitive to testing at the time of extraction. A caries free, asymptomatic, cold and electro sensitive tooth was assumed to be clinically healthy with a vital pulp.

Qualitative findings

Morphological observations primarily comprised the presence or absence of a capsule, hard tissue barrier, inflammation and the type and degree of the latter.

One-week observation

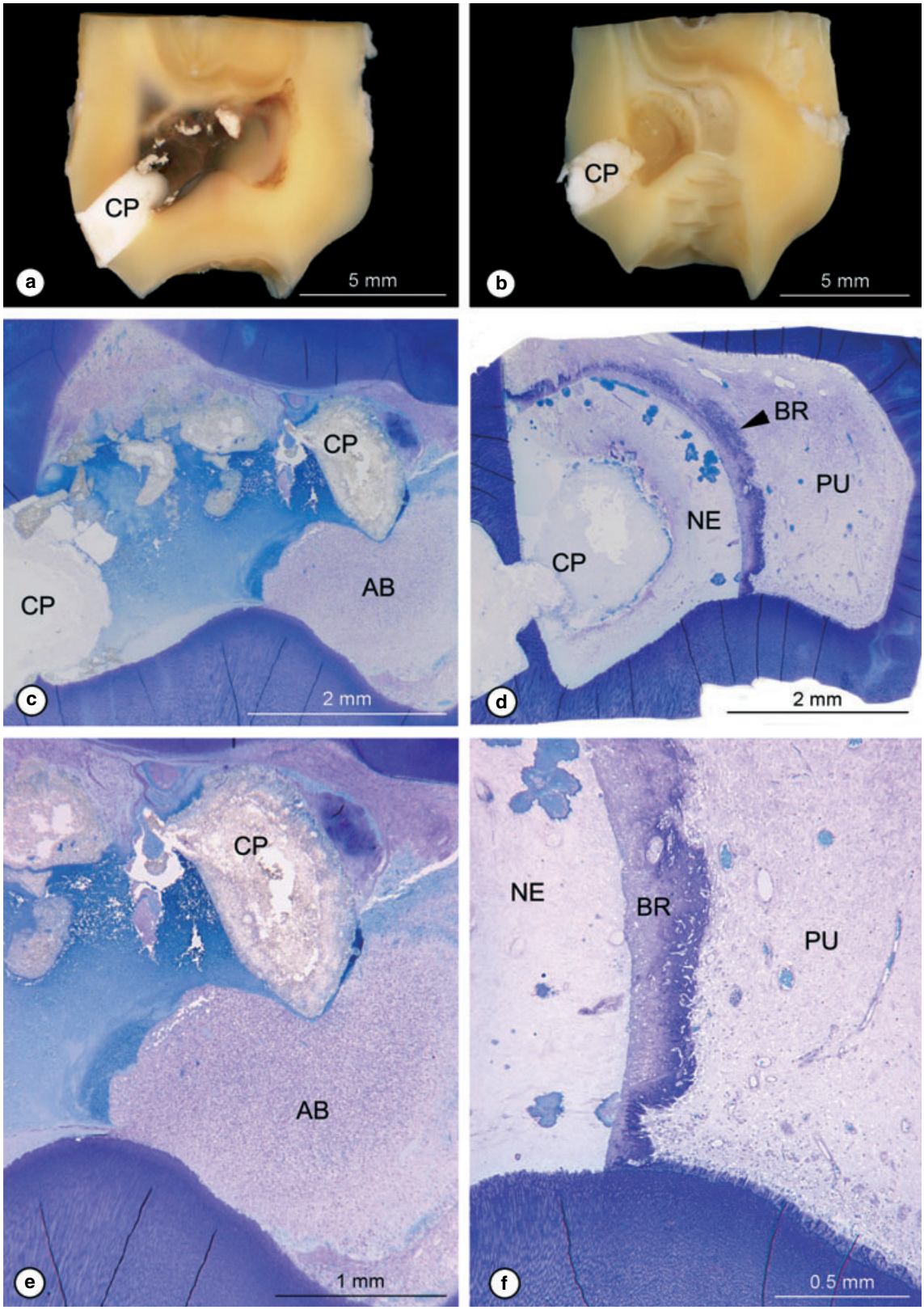
MTA. Five of the six samples (Table 1) were characterized by the presence of a fibrous capsule in contact with the capping material and by the absence of pulpal inflammatory cells as well as of signs of necrosis (Figs 1 and 2). Mild hyperaemia was evident in some of the samples. In one of the samples (specimen 07; Table 1), the exposed pulp tissue was in direct contact with the capping material ('open') and revealed a mild inflammation with mixed inflammatory cell infiltrate.

Dycal®. There was no evidence of hard tissue barrier subjacent to the capping material in all the four specimens (Table 1). A fibrous capsule was evident in one (specimen 21). Two other specimens showed the presence of an inflammatory infiltrate, one with extensive deep (specimen 22; Fig. 3a,c,e) and the other with acute superficial inflammation (specimen 23; Fig. 3b,d,f). There were signs of seepage of Dycal® into the pulp tissue in some of the samples.

One-month observation

MTA. Three of the six specimens (Table 1) revealed a complete hard tissue-bridge lining the pulp (Fig. 4) whilst the other three had a partial bridge. The pulpal cells lining the hard tissue barrier were mostly cuboidal, but also columnar cells were seen in some instances. TEM examination revealed cytoplasmic processes projecting into the bridge (Fig. 5). There was no evidence of necrosis or inflammation between the dentinal bridge and the capping material, and the pulps of all the six samples were free from inflammatory cells.

Dycal®. The pulp was lined by a complete hard tissue bridge only in one of the five specimens (Table 1; specimen 29), that revealed evidence of necrosis coronal to, but absence of inflammation subjacent to the barrier (Fig. 6b,d,f). Three other samples demonstrated a partial hard tissue bridge. In the



remaining one specimen (Table 1; specimen 25) there was neither a capsule nor a hard tissue bridge, but acute pulpal inflammation with a distinct wall of polymorphonuclear leucocytes (PMNs) at the pulp–cap interface. Seepage of Dycal® deep into the pulp tissue could be seen (Fig. 6a,c,e).

Three-month observation

MTA. Four of the five samples (Table 1) revealed a complete hard tissue bridge lining the pulp and one specimen a partial bridge (Figs 7 and 8). The four specimens with complete bridges were devoid of pulpal inflammation. The one with an incomplete bridge (Fig. 8h) showed a small gap between the dentinal wall on the buccal aspect and the hard tissue bridge. This pulpal gap, exposed to the capping material, revealed a mild inflammatory response with localized superficial accumulation of a mixed inflammatory cell population. There was light microscopic evidence of tubule formation at the pulpal aspect of the dentine bridge, that was lined by mostly cuboidal cells (Fig. 7e). TEM examination revealed structures reminiscent of dentinal tubules in early stages of development containing cytoplasm of the lining cells (Fig. 9).

Dycal®. All of the four samples (Table 1) were characterized by the presence of inflammatory cells. Two (specimens 30 and 31) demonstrated an acute inflammatory response with heavy infiltration of PMNs subjacent to the capping material whilst the other two samples had a mixed infiltrate dominated by lymphocytes. One of the specimens with acute inflammatory infiltrate is illustrated in Fig. 10. Presence of microbial infection with bacterial and fungal cells could be recognized in the area of the pulp–cap junction (Fig. 10e). Only partial bridges were observed, and that in only two of the four samples. One of these (specimen 32; Fig. 11) demonstrated a relatively thick incomplete bridge that revealed numerous tunnel defects (Cox *et al.* 1996) at the periphery and gaps on either side. The pulpal gaps without bridge coverage were associated with inflammatory cells. Varying degrees of seepage of the Dycal® into the pulpal tissue was present in all four samples.

Quantitative findings

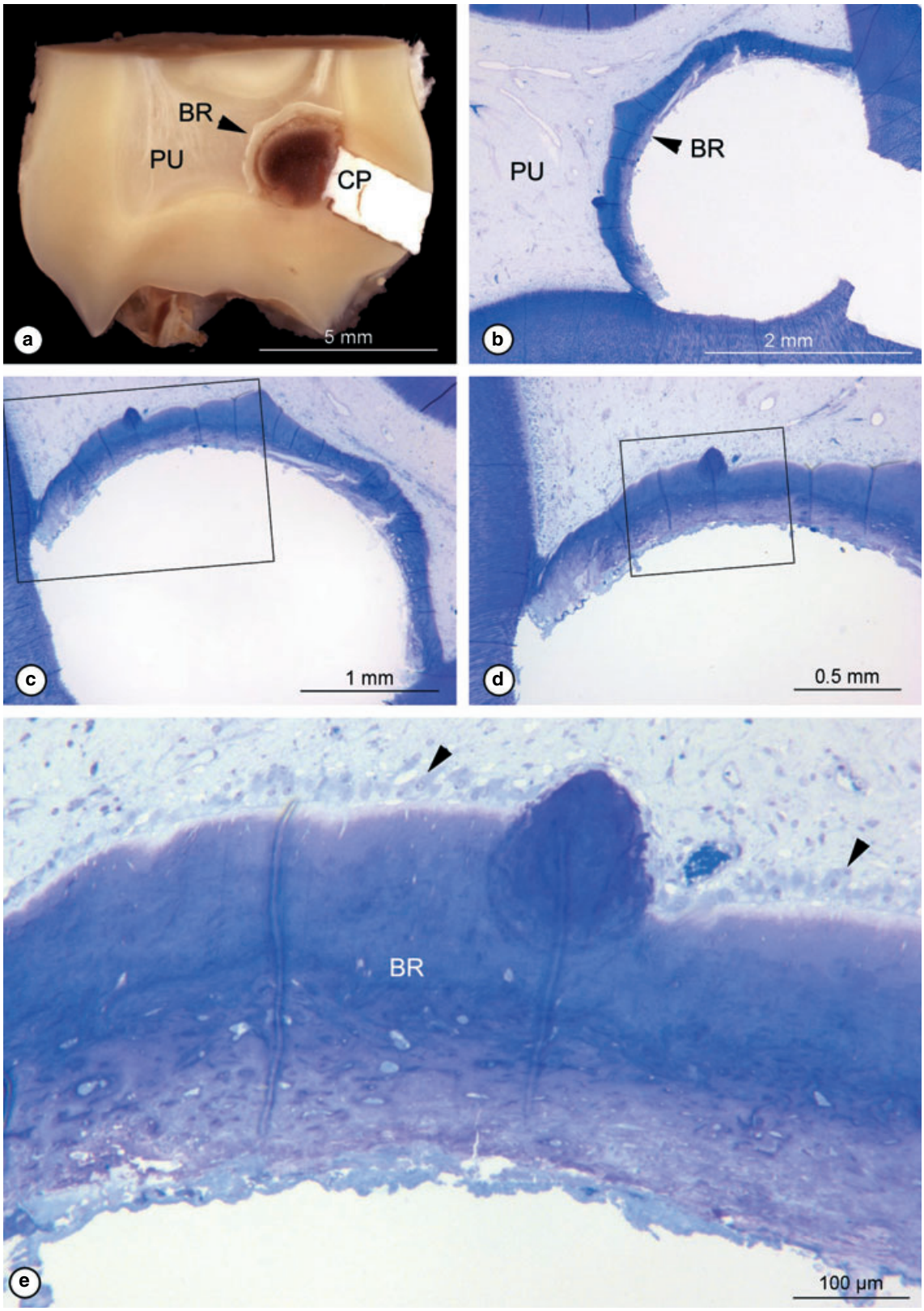
Analysis of pulpal cells

Quantitatively (Table 2), the average number of all pulpal cells per mm^2 increased with increasing period of observation in both the MTA and Dycal® groups, with MTA samples consistently showing lower average cell counts. Whilst the effect resulting from the duration of observation was not significant, the difference between treatments (MTA vs. Dycal®) was highly significant ($P < 0.01$). Fibroblasts represented more than 80% of all cells in all observation periods in the MTA group, but only about 50% in the Dycal® groups ($P < 0.001$). In contrast, proportions of PMNs and round cells (Fig. 12) were significantly ($P < 0.05$ and $P < 0.001$, respectively) lower following MTA as compared with Dycal® treatments. Most probably because of the uneven distribution of 'open', encapsulated and bridged pulp–cap interfaces, sampling significantly affected counts of fibroblasts and infiltrating cells. Particularly within Dycal® specimens, the numbers of PMNs and round cells were consistently higher in 'open' sampling fields as against sites that were encapsulated or bridged (Fig. 12). Macrophages, giant cells and other cellular elements were of rare occurrence in both the MTA and Dycal® specimens (Table 2).

Bridge measurements

The relative length of the bridge (Fig. 13a) increased significantly ($P = 0.001$) with increasing post-treatment period, averages being over 80% after 1 month and almost 100% after 3 months of observation in the MTA group. In the Dycal® specimens, the measure-

Figure 6 Severe inflammatory (a, c, e) and healing (b, d, f) pulpal responses to Dycal® capping after 1 month observation. Distal macrophotographic view (a) of the mesial half of a maxillary right third molar (tooth 18) shows remnants of the restorative and capping material (CP) as a white plug in the cavity preparation and pulp chamber. Note the seepage of the capping material deep into the inflamed pulp tissue. The photomicrographs (c, e) represent a segment of a histological section of the specimen in (a). Note the cavity opening into the pulp chamber, remnants of the capping material (CP) and acute pulpal response and abscess (AB) formation. The right column (b, d, f) presents the mesial view of the distal half of a maxillary left third molar (tooth 28) in (b) and corresponding micrographs showing a healing pulpal (PU) response to the capping material in (d, f). Note the presence of a hard tissue barrier (BR) stretching across the full length of the exposed pulp that is devoid of any signs of inflammation. The interface between the mineralized tissue bridge (BR) and the capping material (CP) is a broad area of necrotic pulp (NE). Original magnifications: a ×5, b ×4.5, c, d ×16, e ×29, f ×45.



ments were significantly ($P < 0.05$) lesser and varied markedly from 0% to over 90%.

Similarly with both treatment procedures, the average thickness of the bridge (Fig. 13b) increased significantly ($P < 0.05$) with increasing observation period. As in the case of the relative bridge length, however, the thickness of the barrier was consistently less variable in the MTA as compared with the Dycal® specimens.

Discussion

This study presents light-microscopic, ultrastructural and quantitative data on pulpal response to direct pulp capping with MTA in healthy human third molars in comparison with $Ca(OH)_2$ (Dycal®) as control. The findings indicate that iatrogenic pulp defects treated with MTA are essentially free from inflammation after 1 week and become covered with compact, dentine-like hard tissue bridges of steadily increasing length and thickness within 3 months following capping. Teeth treated with Dycal® revealed distinctly less consistent formation of a hard tissue barrier that had numerous tunnel defects. Further, the presence of acute and chronic inflammation of the pulp until the longest observation period (3 months) after capping, was a common feature in Dycal® specimens.

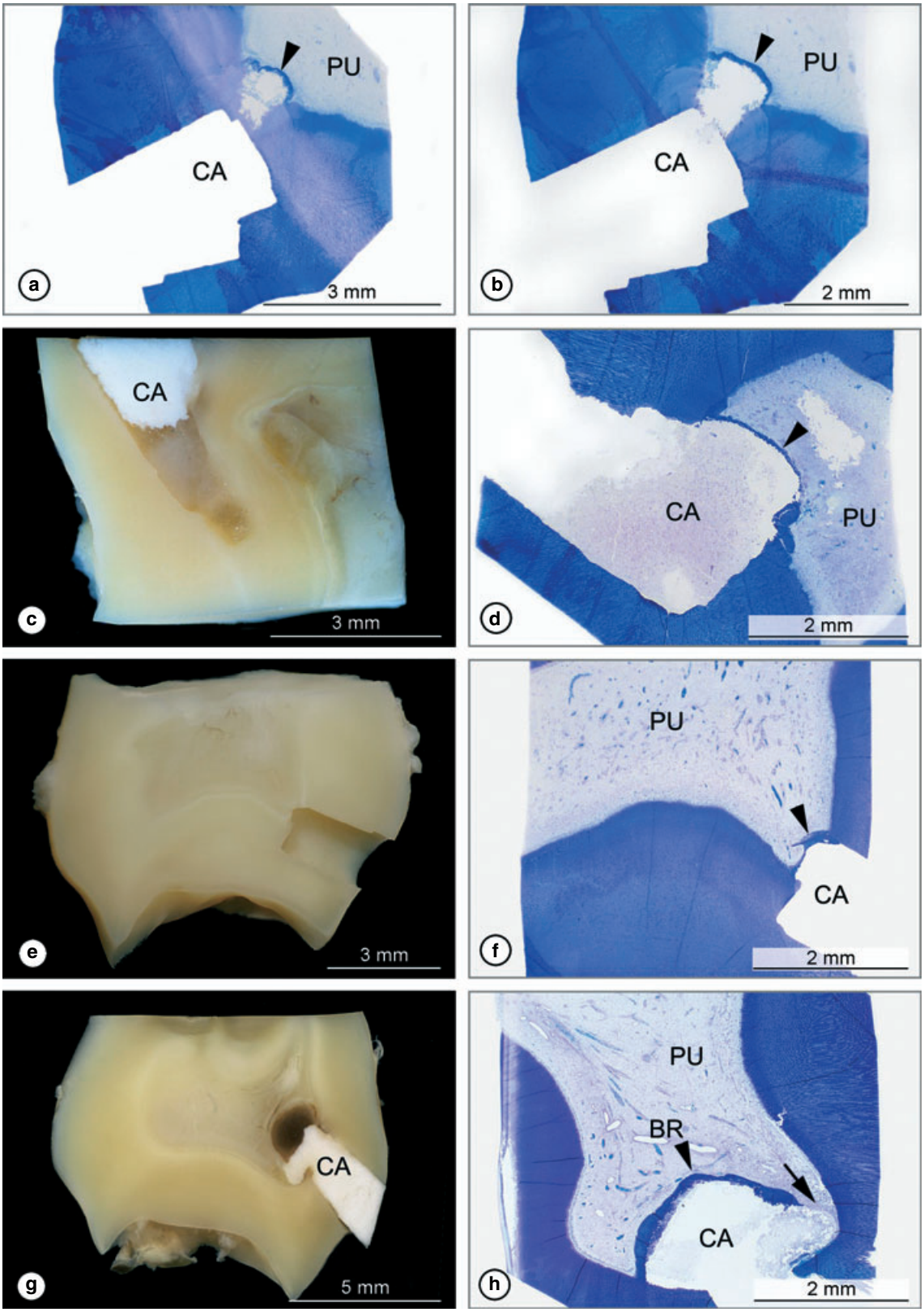
In this study, the precise technique of correlative light and TEM was used so that specific areas in the specimens could be traced in stages from naked eye-level observation to high resolution TEM for details. This enabled observation of the fine morphological features of the pulp–cap interface, particularly of the MTA lining pulpal cells and early tubule formation not previously available. Further, the study enabled comprehensive quantitative data to be obtained on pulpal cells and the bridge formed.

It has been suggested that the sample size should be calculated before starting a trial, based on the anticipated difference in effect that is considered clinically significant (Olsson *et al.* 2006). The 23 patients who participated in this study were recruited over a 2-year period and increasing the number of participants was not possible within the time frame of the study. Recruitment of patients, ethical concerns and time constraints are recognized problems of organizing research of this nature in human subjects and is reflected in recent studies of pulp-capping in which similar or smaller numbers of subjects were recruited (Aeinehchi *et al.* 2003, Caicedo *et al.* 2006, Chacko & Kurikose 2006). It may be pointed out that none of the 23 subjects, whose 35 teeth were included in the study, discontinued with the treatment during the course of the clinical procedures and 30 of the 35 teeth (86%) yielded histological qualitative and quantitative results. Five teeth did not yield results because of clinical and technical reasons and not because of patients 'dropping out' from the study. Therefore, under the time constraints of this study, the numbers were realistically optimal and appropriate statistical evaluation of the quantitative data could be applied.

In the literature, there are a limited number of animal studies on pulp capping in which MTA has been compared with $Ca(OH)_2$ in monkeys (Pitt Ford *et al.* 1996), dogs (Tziafas *et al.* 2002, Asgary *et al.* 2006) and rats (Salako *et al.* 2003). These studies consistently demonstrated more hard tissue bridge formation and less inflammation in the MTA group compared with the $Ca(OH)_2$ control over a 2–5 month period. The observed effects in animals may not be directly applicable to those in humans, and usage trials on human teeth should be mandatory for clinical application. Amongst the tests recognized by the International Organization for Standardization (ISO 1997) to evaluate a dental material for routine therapeutic usage, the application of the material in human teeth is the last usage test before recommendation for clinical application.

There appear to be four histological reports on human teeth in which MTA has been used as a direct pulp–capping agent. One of them (Aeinehchi *et al.* 2003), a preliminary study, compared MTA with Dycal® in human permanent teeth over three time intervals. In the study, however, a sizable number of patients (6) discontinued the treatment prior to extraction of the teeth with the result that some time

Figure 7 Pulpal response to mineral trioxide aggregate (MTA) capping after 3 months observation. Distal macrophotographic view (a) of the mesial half of a maxillary left third molar (tooth 28) shows the remnants of the restorative and capping material (CP) and a distinct hard tissue bridge (BR) across the exposed pulp (PU). The photomicrograph (b) is part of a histological section of the specimen in (a). Note the mineralized hard tissue barrier (BR), stretching across the full length of the exposed pulp (b, c). The rectangular areas demarcated in (c) and (d) are magnified in (d) and (e) respectively. Note the cuboidal pulpal cells (arrowheads) lining the bridge (BR) and absence of pulpal inflammation in (e). Original magnifications: a ×6, b ×8, c ×23, d ×200.



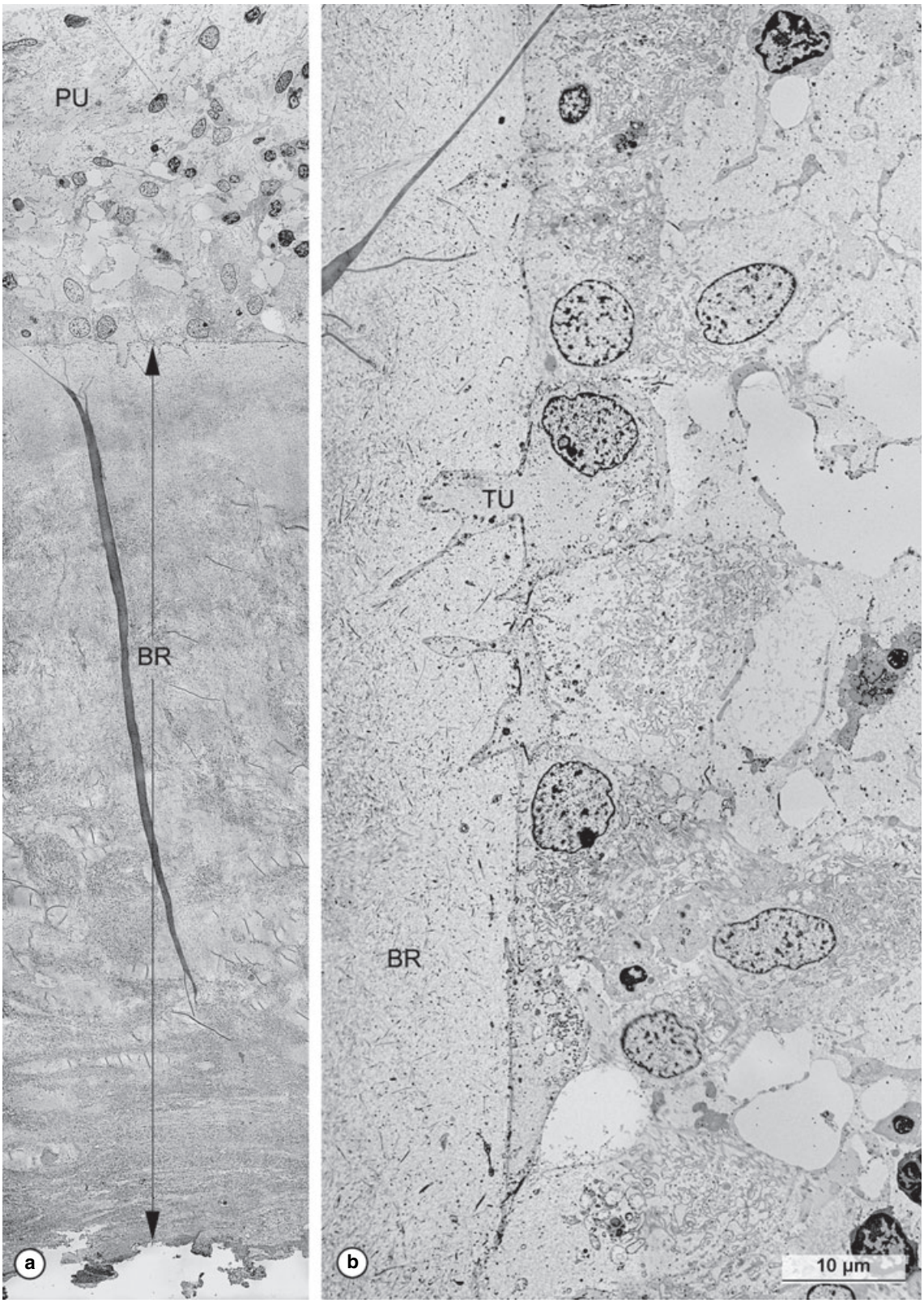
intervals had only two teeth available for histological examination. Therefore, no statistical analysis could be carried out. In another study (Iwamoto *et al.* 2006) on human third molars ($n = 48$) the pulp capping ability of white MTA was compared with that of Dycal®. Forty-five of the 48 teeth were histologically examined using paraffin technology after a single observation period of about 4.5 months. Twenty of the 22 MTA-treated and 18 of the 23 Dycal-treated teeth 'developed a bridge' and it was concluded that '…MTA was as effective a pulp capping material as the control calcium hydroxide'. However, no data were presented on the width and relative length (in relation with the pulpal opening) of the hard tissue barrier formed. Therefore, it is not clear how many of the teeth in the MTA and Dycal groups developed complete bridges. On the other hand, the present study reported here, with three observation periods, qualitative and quantitative data on the bridge and pulpal inflammation provides a different outcome, with MTA clearly performing superior to Dycal® as a pulp–capping agent. In the remaining two studies MTA had been used in human primary teeth, for direct pulp capping (Caicedo *et al.* 2006) and after 'pulpal amputation' (Caicedo *et al.* 2006, Chacko & Kurikose 2006). In the latter procedure, the coronal pulp was removed to the orifice of the root canal. Based on purely qualitative histological evaluation these studies demonstrated less inflammation and consistent bridge formation with MTA compared with $Ca(OH)_2$. The present study not only corroborates the positive qualitative observations in the three previous publications but also provides fine structural and quantitative data on a larger, statistically reliable number of specimens in the trial (MTA) and the control (Dycal®) groups.

Human studies using healthy teeth are without possible confounding factors such as caries and coronal restorations. However, pulpal exposures are more likely to occur in carious teeth possibly complicated with pulpal inflammation. Microbes and their products interfere with the pulpal response to capping materials. Therefore, the relevance of this and other reported studies conducted on healthy human teeth may be clinically limited (Olsson *et al.* 2006). It would be of long-term interest, if direct pulp capping studies on human teeth using MTA are performed on carious teeth with vital pulps that might be exposed during therapeutic procedures and the pulpal response studied histologically. It has been suggested that healing of the dental pulp was not significantly affected by the type of medicament but rather on the ability of the material to prevent microleakage (Tobias *et al.* 1982, Cox *et al.* 1987). In this study, all the cavities were surface-sealed with zinc oxide–eugenol to prevent microbial leakage; however, pulpal inflammation was present in most of the Dycal® samples.

With respect to pulpal cellular response, the MTA performed well in the current study. Superficial local accumulation of inflammatory cells was observed in only one sample out of 17. The pulps of the MTA specimens were dominated in all three observation periods by fibroblasts and almost lacked infiltrating inflammatory cells. It appears that, under the conditions of this study, MTA has excellent sealing properties and prevents microleakage and pulpal inflammation by providing a predictable secondary barrier under the surface seal. This may be important clinically as it is not possible to place a surface seal such as zinc oxide eugenol cement as a permanent restoration. Permanent restorations such as amalgam and composite restorations may allow increased microleakage. This means that in a clinical situation the seal provided by the capping material should probably be more leakage proof than the one achieved in this study.

The Dycal® results were far less satisfactory with nine out of 13 samples demonstrating pulpal inflammation. Acute inflammation with heavy PMN infiltration was observed in five of the nine samples suggesting the presence of microbes and microleakage. The

Figure 8 Overviews of pulpal response to mineral trioxide aggregate (MTA) capping after 3 months observation in four remaining specimens. Bucco-lingual photomicrographic views of two consecutive serial sections (a, b), several micrometers apart, of a maxillary left third molar (tooth 28) shows the cavity preparation (CA), a distinct hard tissue bridge (arrowheads) and a healthy pulp (PU). (c, d) are macro- and microphotographs of a mandibular left third molar (tooth 38) with cavity preparation (CA), complete hard tissue bridge (arrowhead in d) and a healthy pulp (PU). (e, f) are macro- and microphotographs of a maxillary right third molar (tooth 18) with cavity preparation (CA), complete hard tissue bridge (arrowhead in f) and a healthy pulp (PU). (g, h) are macro- and microphotographs of a maxillary right third molar (tooth 18) with cavity preparation (CA), hard tissue bridge (BR) and healthy pulp (PU) in (h). This was the only specimen in the MTA-capped group at 3 months observation that showed a bridge with a small gap (arrow in h) on the vestibular side of the pulp. Original magnifications: a, b, c, f ×10, d, h ×14, e, g ×6.



routine observation of microbes in histological sections using the light microscope is often not predictable (Nair 1987). Nevertheless, in one specimen the acute inflammation was associated with mixed microbial infection consisting of bacterial and fungal organisms. Therefore, leakage of microbes and/or antigenic products may be the cause of the acute inflammatory reaction and Dycal® seems to fail to provide an effective barrier against such leakage. Four Dycal® samples were associated with infiltration of predominantly chronic inflammatory cells that is indicative of a cause other than leakage of viable microbes. The presence of chronic inflammatory cells suggest that a component of Dycal® produced a sustained irritation to the pulp which was not evident in the more biocompatible MTA. Whether this inflammation would resolve over the long-term period is unknown. Further, Dycal® was found to be technically much more difficult to apply over the exposed pulp than MTA. This was evident in small particles of the Dycal® material being present within the pulpal tissue in some samples. It has also been suggested that Dycal® may be absorbed to some extent by macrophages, allowing antigen passage into the pulp that can maintain at least part of the low grade inflammatory reaction (Faraco & Holland 2001).

With regard to bridge formation the MTA performed much better than Dycal®. Within the confines of this study, three out of six in the 1-month and four of five in the 3-month samples of MTA demonstrated complete bridge formation. Mostly cuboidal cells lined the bridge. The presence in some specimens of columnar cells with polarized nuclei and cytoplasm rich in rough endoplasmic reticulum projecting into invaginations of the bridge is clearly indicative of the formation of odontoblast-like cells and initiation of tubular dentine. Particularly the bridges formed at 3 months of observation were uniformly thick and continuous with the surrounding dentine walls. Further, the presence of relatively few visible tunnel defects (Cox *et al.* 1996) indicates the compactness of the hard tissue barrier formed with MTA, which points to a 'good quality' bridge formed. The biological mechanism by which MTA induces dentine bridge formation is currently unknown. The predictable formation of a quality hard tissue barrier subjacent to MTA is likely to be multifactorial, involving its sealing ability (Torabinejad *et al.* 1993, Wu *et al.* 1998), biocompatibility (Mitchell *et al.* 1999, Keiser *et al.* 2000) and production of an alkaline pulpal environment (Camilleri *et al.* 2005). It has been shown *in vitro* that the MTA stimulates certain cytokine release in human osteoblasts and may provide an active role in hard tissue formation (Koh *et al.* 1995, 1998).

Bridge formation subjacent to Dycal® was also evident but was unpredictable with varying thickness and numerous tunnel defects (Cox *et al.* 1996). This is indicative of a 'poor quality' bridge formed in Dycal® specimens. It has been demonstrated that the origin of the calcium forming the bridge was not from the medicament (Sciaky & Pisanti 1960, Pisanti & Sciaky 1964). The alkalinity of $Ca(OH)_2$ may dampen inflammatory responses and provide a relatively sterile environment for repair to occur (Goldberg & Smith 2004). Therefore, the action of Dycal® may be relatively non-specific and MTA may be more effective in this role.

During the time frame of this study, there was only limited histological evidence of tubular dentine, although odontoblast-like cell formation and early tubule development were observed in several specimens of the MTA capped teeth. The longest of the three observation periods in this study was 3 months, as the aim was to establish the short-term pulpal response after capping with MTA or Dycal®. In addition, there were ethical considerations in delaying further the extraction of the teeth; the maximum waiting time from assessment to extraction was 4 months. It would be of interest if longer-time intervals of at least 1 year could be examined to see whether tubular dentine lined with well differentiated odontoblast-like cells is formed, and to assess the long-term thickness and quality of the bridge. In addition, it would be of value to determine the response of the soft tissue to MTA as a capping material in comparison with Dycal® after 1 year.

Handling characteristics are important when considering any material for clinical use. Hard setting $Ca(OH)_2$ cements such as Dycal® are hydrophobic and require a completely dry cavity, particularly with no intervening blood, for effective placement. On the other hand, MTA requires moisture to set and is

Figure 9 Correlative composite low magnification (a) transmission electron micrograph of the bridge (BR) and subjacent pulp (PU) shown in Fig. 7. The bridge–pulp interface in (a) is magnified and turned 90° clockwise in (b). The bridge (BR) is lined on the pulpal side by cuboidal cells. Note the cytoplasmic projections of the cells into the bridge, reminiscent of developing dentinal tubules (TU). Original magnification: a ×450, b ×21 000.

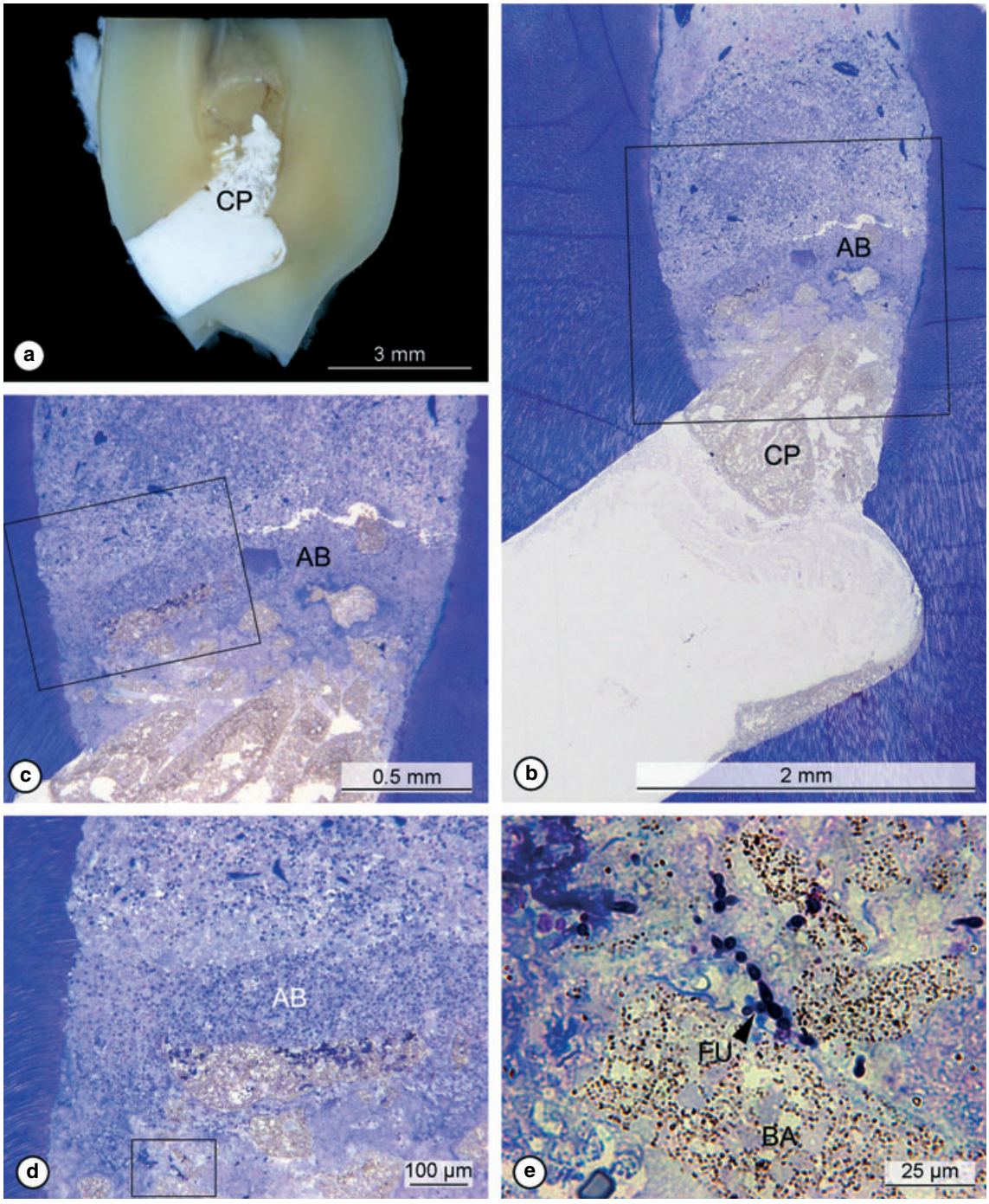


Figure 10 Acute pulpal response to Dycal® capping after 3 months observation. Mesial macrophotographic view (a) of the distal half of a maxillary left third molar (tooth 28) shows the remnants of the restorative and capping material (CP) in the cavity preparation and pulp chamber. Note the cavity in (b) opening into the pulp chamber, remnants of the capping material (CP), and acute inflammatory pulpal response (AB). (c, d, e) are higher magnifications of the rectangular areas demarcated in (b), (c) and (d) respectively. The acute inflammation (AB) is associated with mixed microbial infection consisting of bacterial (BA) and fungal (FU) organisms that are visible at the interface between the capping material and exposed pulp (e). Original magnifications: a ×8, b ×27, c ×42, d 90, e ×560.

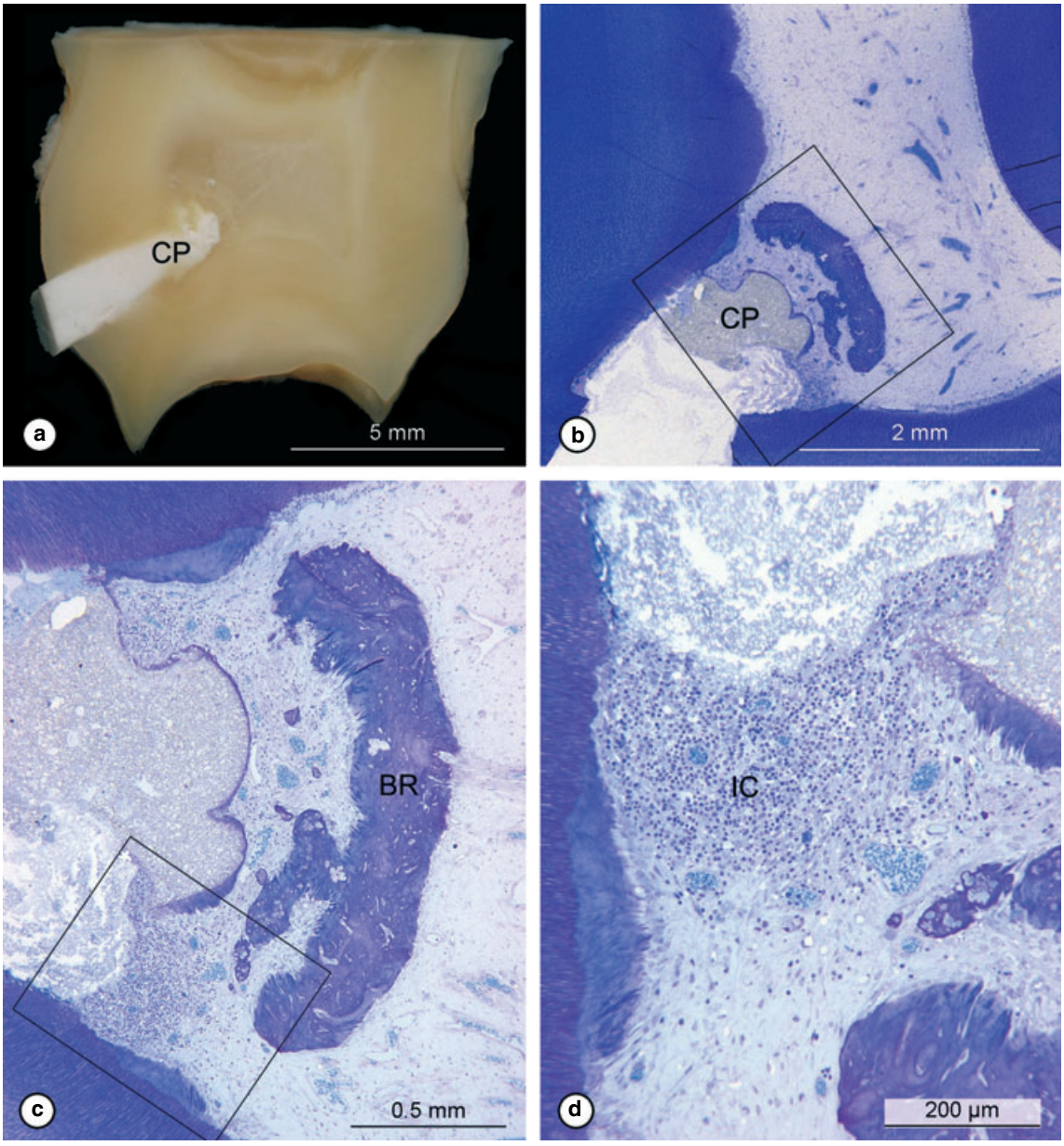


Figure 11 Mixed pulpal response to Dycal® capping after 3 months observation. Distal macrophotographic view (a) of the mesial half of a maxillary right third molar (tooth 18) shows the remnants of the restorative and capping material (CP) as a white plug in the cavity preparation and pulp chamber. The photomicrograph (b) is a segment of a histological section of the specimen in (a). Note the cavity opening into the pulp chamber and remnants of the capping material (CP). The rectangular areas demarcated in (b) and (c) are magnified in (c) and (d) respectively. The distinct but incomplete hard tissue bridge (BR in c) reveals gaps on either side with infiltrate of chronic inflammatory cells (IC in d). Original magnifications: a ×6, b ×18, c ×46, d ×120.

relatively easy to use in contact with a moist tissue such as the pulp. It was difficult to achieve a dry field in this study because of the dampness of the pulp even after any bleeding had been arrested. This made Dycal® placement a more time consuming and technically demanding procedure than the equivalent MTA procedure.

The results of this study allow the conclusion that MTA is clinically easier to use and results in less pulpal inflammation and more predictable hard tissue barrier

Table 2 Means and ranges (min.–max.) of cell counts at the capped pulpal front and significance of effects resulting from the treatment (T; MTA vs. Dycal®), the observation period (O), and the sampling subjacent to open, encapsulated and bridged pulp–cap interfaces (F)

			Significance[a] of factors		
Cell type (units)	Mean	Min.–max.	T	O	F
All cells (n/mm^2)			**	NS	*
MTA					
1 week	808	328–2458			
1 month	851	725–970			
3 months	1281	723–2285			
Dycal®					
1 week	1775	760–3940			
1 month	1988	703–3528			
3 months	2682	978–4080			
Fibroblasts (%)			***	NS	*
MTA					
1 week	84.4	48.5–94.5			
1 month	89.8	86.4–94.0			
3 months	82.7	62.8–89.2			
Dycal®					
1 week	53.4	7.8–81.1			
1 month	54.6	19.7–80.1			
3 months	49.2	14.3–76.0			
PMNs (Fig. 12a)			*	NS	NS
Round cells (Fig.12b)			***	NS	*
Macrophages (%)			ns	*	*
MTA					
1 week	0.1	0.0–0.9			
1 month	0.0				
3 months	0.03	0.0–0.2			
Dycal®					
1 week	0.8	0.0–2.0			
1 month	0.04	0.0–0.2			
3 months	0.08	0.0–0.2			
Giant cells (%)			NS	NS	NS
MTA					
1 week	0.0				
1 month	0.0				
3 months	0.0				
Dycal®					
1 week	0.2	0.0–0.8			
1 month	0.0				
3 months	0.04	0.0–0.2			
Other cells (%)			*	NS	NS
MTA					
1 week	0.7	0.0–4.0			
1 month	0.5	0.0–1.4			
3 months	1.4	0.0–2.7			
Dycal®					
1 week	0.1	0.0–0.4			
1 month	0.0				
3 months	0.0				

[a]NS: $P > 0.05$; *$0.05 \geq P > 0.01$; **$0.01 \geq P > 0.001$; ***$P \leq 0.001$.

formation than Dycal®. Therefore, MTA or equivalent products should be the material of choice for direct pulp capping procedures as against the continuing recommendation (Olsson *et al.* 2006) of $Ca(OH)_2$ as the gold standard for such treatments.

Acknowledgements

The authors are indebted to Mrs Margrit Amstad-Jossi for skilful technical assistance.

References

Aeinehchi M, Eslami B, Ghanbariha M, Saffar AS (2003) Mineral trioxide aggregate (MTA) and calcium hydroxide as pulp capping agents in human teeth: a preliminary report. *International Endodontic Journal* **36**, 225–31.

Asgary S, Parirokh M, Eghbal MJ, Ghoddusi J, Eskandarizadeh A (2006) SEM evaluation of neodentinal bridging after direct pulp protection with mineral trioxide aggregate. *Australian Endodontic Journal* **32**, 26–30.

Bennett HS, Luft JH (1959) s-Collidine as a basis for buffering fixatives. *Journal of Biophysics, Biochemistry and Cytology* **6**, 113–4.

Caicedo R, Abbott PV, Alongi DJ, Alarcon MY (2006) Clinical, radiographic and histological analysis of the effects of mineral trioxide aggregate used in direct pulp capping and pulpotomies of primary teeth. *Australian Dental Journal* **51**, 297–305.

Camilleri J, Montesin FE, Di Silvio L, Pitt Ford TR (2005) The chemical constitution and biocompatibility of accelerated Portland cement for endodontic use. *International Endodontic Journal* **38**, 834–42.

Chacko V, Kurikose S (2006) Human pulpal response to mineral trioxide aggregate (MTA): a histologic study. *Journal of Clinical Pediatric Dentistry* **30**, 203–10.

Cox CF, Keall CL, Keall HJ, Ostro E, Bergenholtz G (1987) Biocompatibility of surface-sealed dental materials against exposed pulps. *Journal of Prosthetic Dentistry* **57**, 1–8.

Cox CF, Sübay RV, Ostro E, Suzuki S, Suzuki SH (1996) Tunnel defects in dentine bridges: their formation following pulp capping. *Operative Dentistry* **21**, 4–11.

Cvek M, Granath L, Cleaton-Jones P, Austin J (1987) Hard tissue barrier formation in pulpotomized monkey teeth capped with cyanoacrylate or calcium hydroxide for 10 and 60 minutes. *Journal of Dental Research* **66**, 1166–74.

ESE (2006) Quality guidelines for endodontic treatment: consensus report of the European Society of Endodontology. *International Endodontic Journal* **39**, 921–30.

Faraco IM, Holland R (2001) Response of the pulp of dogs to capping with mineral trioxide aggregate or a calcium hydroxide cement. *Dental Traumatology* **17**, 163–6.

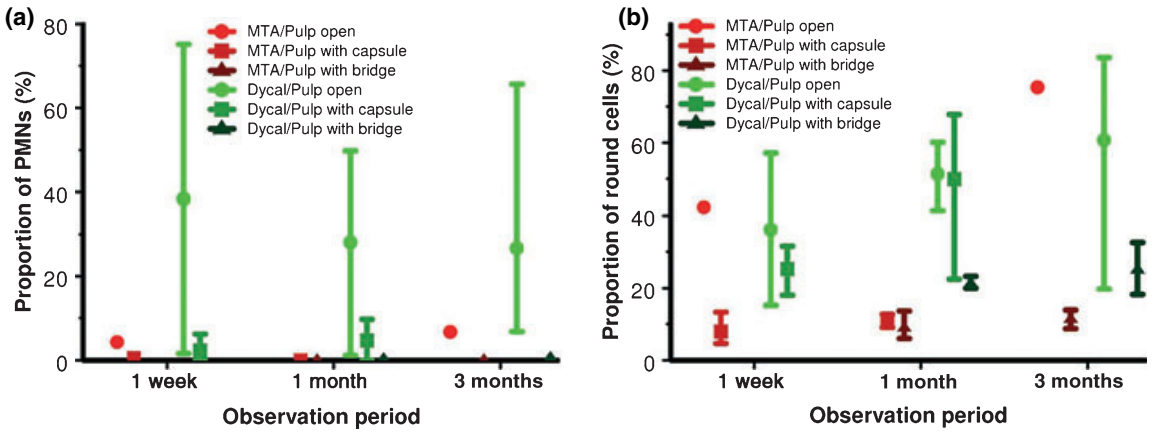


Figure 12 Graphic representations of proportions of PMNs (a) and round cells (b; in % of all cells present) as a function of the observation period. Means (dots and squares) and ranges (vertical bars) are displayed for pulp areas that were in direct contact with the capping material (open) and covered by a fibrous capsule or hard tissue bridge in specimens treated with mineral trioxide aggregate (MTA) and Dycal®.

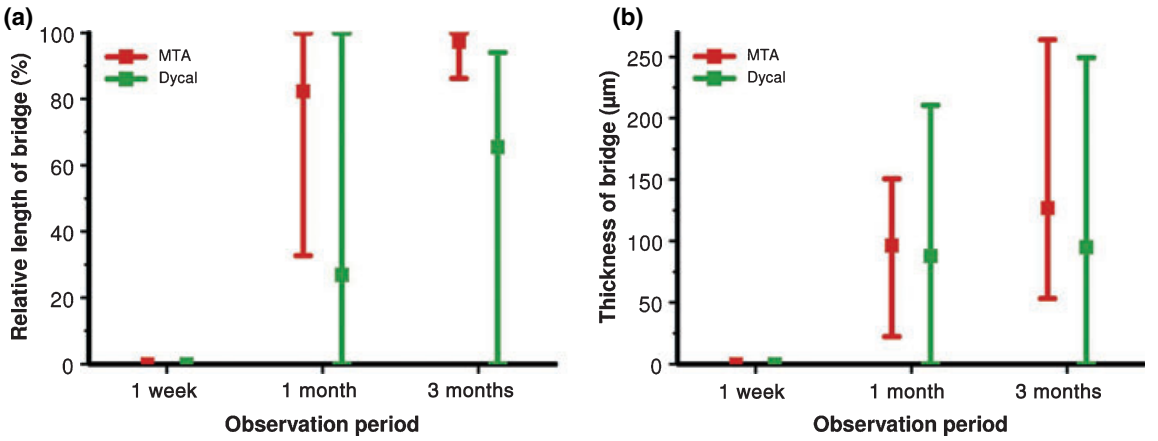


Figure 13 Graphic representations of the relative length (a; in % of the total pulp defect length) and thickness (b; in µm) of the hard tissue bridges. Means (dots and squares) and ranges (vertical bars) are plotted as a function of the observation period in specimens treated with mineral trioxide aggregate (MTA) and Dycal®.

Farsi N, Alamoudi N, Balto K, Mushayt A (2005) Success of mineral trioxide aggregate in pulpotomized primary molars. *Journal of Clinical Pediatric Dentistry* **29**, 307–11.

Farsi N, Alamoudi N, Balto K, Mushayt A (2006) Clinical assessment of mineral trioxide aggregate (MTA) as direct pulp capping in young permanent teeth. *Journal of Clinical Pediatric Dentistry* **31**, 72–6.

Fraska JM, Parks VR (1965) A routine technique for double staining ultrathin sections using uranyl and lead salts. *Journal of Cell Biology* **25**, 157–61.

Glass RI, Zahnder HA (1949) Pulp healing. *Journal of Dental Research* **28**, 97–107.

Goldberg M, Smith AJ (2004) Cells and extracellular matrices of dentin and pulp: a biological basis for repair and tissue engineering. *Critical Reviews in Oral Biology and Medicine* **15**, 13–27.

Hermann BW (1930) Dentinobliteration der wurzelkanäle nach behandlung mit calcium. *Zahnärztliche Rundschau* **39**, 888–9.

Hermann BW (1936) *Biologische Wurzelbehandlung*. Frankfurt am Main: Kramer.

Holland R, Souza VMJN, Otoboni Filho JA, Bernabé PFE, Dezan E Jr (1999a) Reaction of rat connective tissue to implanted dentine tubes filled with mineral trioxide aggregate or calcium hydroxide. *Journal of Endodontics* **25**, 161–6.

Holland R, Souza V, Nery MJ, Otoboni Filho JA, Bernabé PFE, Dezan E Jr (1999b) Reaction of dogs' teeth to root canal filling with mineral trioxide aggregate or glass ionomer sealer. *Journal of Endodontics* **25**, 728–30.

ISO (1997) *Dentistry – Preclinical Evaluation of Biocompatibility of Medical Devices Used in Dentistry. Test Methods for Dental Materials*. International Organization for Standardization, Technical report, Geneva, 7405.

Iwamoto CE, Adachi E, Pameijer CH, Barnes D, Romberg EE, Jefferies S (2006) Clinical and histological evaluation of white ProRoot MTA in direct pulp capping. *American Journal of Dentistry* **19**, 85–90.

Karnovsky MJ (1965) A formaldehyde glutaraldehyde fixative of high osmolarity for use in electron microscopy. *Journal of Cell Biology* **27**, 137A–9A.

Keiser K, Johnson CC, Tipton DA (2000) Cytotoxicity of mineral trioxide aggregate using human periodontal ligament fibroblasts. *Journal of Endodontics* **26**, 288–91.

Koh ET, Pitt Ford TR, Torabinejad M, McDonald F (1995) Mineral trioxide aggregate stimulates cytokine production in human osteoblasts. *Journal of Bone and Mineral Research* **10S**, S406.

Koh ET, McDonald F, Pitt Ford TR, Torabinejad M (1998) Cellular response to mineral trioxide aggregate. *Journal of Endodontics* **24**, 543–7.

Langeland K, Dowden WE, Tronstad L, Langeland LK (1973) Human pulp changes of iatrogenic origin. In: Siskin M, ed. *The Biology of the Human Dental Pulp*, pp. 122–59. St. Louis, CA: C.V. Mosby.

Lee SJ, Monsef M, Torabinejad M (1993) Sealing ability of a mineral trioxide aggregate for repair of lateral root perforations. *Journal of Endodontics* **19**, 541–4.

Maroto M, Barbería E, Planells P, Godoy FG (2005) Dentin bridge formation after mineral trioxide aggregate (MTA) pulpotomies in primary teeth. *American Journal of Dentistry* **18**, 151–4.

Maroto M, Barbería E, Vera V, Godoy FG (2006) Dentin bridge formation after white mineral trioxide aggregate (white MTA) pulpotomies in primary teeth. *American Journal of Dentistry* **19**, 75–9.

Mitchell PJC, Pitt Ford TR, Torabinejad M, McDonald F (1999) Osteoblast biocompatibility of mineral trioxide aggregate. *Biomaterials* **20**, 167–73.

Nair PNR (1987) Light and electron microscopic studies of root canal flora and periapical lesions. *Journal of Endodontics* **13**, 29–39.

Nyborg H (1955) Healing process in the pulp on capping. *Acta Odontologica Scandinavica* **13**, 1–130.

Olsson H, Petersson K, Rohlin M (2006) Formation of hard tissue barrier after pulp capping in humans: a sytematic review. *International Endodontic Journal* **39**, 429–42.

Pisanti S, Sciaky I (1964) Origin of calcium in the repair wall after pulpal exposure in the dog. *Journal of Dental Research* **43**, 641–4.

Pitt Ford TR, Roberts GJ (1991) Immediate and delayed direct pulp capping with the use of a new visible light-cured calcium hydroxide preparation. *Oral Surgery Oral Medicine and Oral Pathology* **71**, 338–42.

Pitt Ford TR, Torabinejad M, McKendry J, Hong C-U, Kariyawasam SP (1995) Use of mineral trioxide aggregate for repair of furcal perforations. *Oral Surgery Oral Medicine and Oral Pathology* **79**, 756–62.

Pitt Ford TR, Torabinejad M, Abedi HR, Backland LK, Kariyawasam SP (1996) Using mineral trioxide aggregate as a pulp–capping material. *Journal of American Dental Association* **127**, 1491–4.

Rebel HH (1922) Über die ausheilung der freigelegten pulpa. *Deutsche Zahnheilkunde* **55**, 3–83.

Salako N, Joseph B, Ritwik P, Salonen J, John P, Junaid TA (2003) Comparison of bioactive glass, mineral trioxide aggregate, ferric sulfate, and formocresol as pulpotomy agents in rat molar. *Dental Traumatology* **19**, 314–20.

Schröder U (1972) Evaluation of healing following experimental pulpotomy of intact human teeth and capping with calcium hydroxide. *Odontologisk Revy* **23**, 329–40.

Schröder U (1973a) Reaction of the human dental pulp to experimental pulpotomy and capping with calcium hydroxide. *Odontologisk Revy* **24**(Suppl. 25), 1–33.

Schröder U (1973b) Effect of an extra-pulpal blood clot on healing following experimental pulpotomy and capping with calcium hydroxide. *Odontologisk Revy* **24**, 257–67.

Schröder U (1978) A 2-year follow-up of primary molars pulpotomized with a gentle technique and capped with calcium hydroxide. *Scandinavian Journal of Dental Research* **86**, 273–8.

Sciaky I, Pisanti S (1960) Localization of calcium placed over amputated pulps in dogs' teeth. *Journal of Dental Research* **39**, 1128–32.

Stanley HR, Lundy T (1972) Dycal therapy for pulp exposure. *Oral Surgery Oral Medicine and Oral Pathology* **34**, 818–27.

Tobias RS, Plant CG, Browne RM (1982) Reduction in pulpal inflammation beneath surface-sealed silicates. *International Endodontic Journal* **15**, 173–80.

Torabinejad M, Watson TF, Pitt Ford TR (1993) Sealing ability of mineral trioxide aggregate when used as a root end filling material. *Journal of Endodontics* **19**, 591–5.

Torabinejad M, Hong CU, Lee SJ, Monsef M, Pitt Ford TR (1995) Investigation of mineral trioxide aggregate for root-end filling in dogs. *Journal of Endodontics* **21**, 603–8.

Tronstad L (1974) Reaction of the exposed pulp to dycal treatment. *Oral Surgery Oral Medicine and Oral Pathology* **38**, 945–53.

Tziafas D, Pantelidou O, Alvanou A, Belibasakis G, Papadimitriou S (2002) The dentinogenic effect of mineral trioxide aggregate (MTA) in short-term capping experiments. *International Endodontic Journal* **35**, 245–54.

Venable JH, Coggeshall R (1965) A simplified lead citrate stain for use in electron microscopy. *Journal of Cell Biology* **25**, 407–8.

Weibel ER (1970) An automatic sampling stage microscope for stereology. *Journal of Microscopy* **91**, 1–18.

Wu MK, Kontakiotis EG, Wesselink PR (1998) Long-term seal provided by some root-end filling materials. *Journal of Endodontics* **24**, 557–60.